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(54) NEW POLYPEPTIDE

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a polypeptide useful for screening for and/or developing an agent for treating, preventing, and/or diagnosing a disease related to the activation of NF-κB, a DNA encoding the polypeptide, an antisense DNA/RNA of the DNA the gene therapy using the DNA, an antibody recognizing the polypeptide, a modified polypeptide derived from the preceding polypeptide and having an enhanced activity, a dominant negative variant of the polypeptide, and methods for utilizing these.
SOLUTION: A polypeptide activating NF-κB is identified to produce a DNA encoding the polypeptide and an antibody recognizing the polypeptide. These can be utilized fro screening for a medicine for and diagnosing a disease related to the activation of NF-κB.

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CLAIMS

[Claim(s)]
[Claim 1] The polypeptide which has the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5.
[Claim 2] The polypeptide which has the activity which one or more amino acid consists [activity] of deletion and amino acid sequences permuted and/or added in the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and raises the activity of NF-kappa B.
[Claim 3] The polypeptide which has the activity which raises the activity of NF-kappa B, including the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and the amino acid sequence which has 60% or more of homology.
[Claim 4] DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 5] DNA which has the base sequence expressed with either of the array numbers 6-10.
[Claim 6] DNA which carries out the code of the polypeptide which has the activity which it is [activity] DNA according to claim 4 or 5 and DNA hybridized under stringent conditions, and raises the activity of transcription factor NF-kappa B.
[Claim 7] The recombinant vector which includes DNA of a publication in any 1 term of claims 4-6 at a vector, and is obtained.
[Claim 8] The recombinant vector which includes in a vector RNA which becomes any 1 term of claims 4-6 from DNA of a publication, and a homologous array, and is obtained.
[Claim 9] The recombinant vector according to claim 8 whose RNA is a single strand.
[Claim 10] The transformant which holds a recombinant vector according to claim 7.
[Claim 11] The transformant according to claim 10 whose transformant is a transformant chosen from the group which consists of a microorganism, an animal cell, a plant cell, and an insect cell.
[Claim 12] The transformant according to claim 11 whose microorganism is a microorganism belonging to an Escherichia group.
[Claim 13] The transformant according to claim 11 whose animal cell is an animal cell chosen from a mouse myeloma cell, a rat myeloma cell, a mouse hybridoma cell, a CHO cell, a BHK cell, an African green monkey kidney cell, a Namalwa cell, Namalwa KJM-1 cell, a Homo sapiens embryo kidney cell, and a Homo sapiens leukemic cell.
[Claim 14] The transformant according to claim 11 whose insect cell is an insect cell chosen from the ovarian cell of Spodoptera frugiperda, the ovarian cell of Trichoplusia ni, and the ovarian cell of a silkworm.
[Claim 15] The transformant according to claim 10 whose transformant is a nonhuman transgenic animal or a transgenic plant.
[Claim 16] The manufacture approach of this polypeptide which cultivates a transformant given in any 1 term of claims 10-14 to a culture medium, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into a culture, and is characterized by extracting this polypeptide from this culture.
[Claim 17] The manufacture approach of this polypeptide which breeds the nonhuman transgenic

animal which holds a recombinant DNA according to claim 7, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into this animal, and is characterized by extracting this polypeptide from the inside of this animal.
[Claim 18] The manufacturing method according to claim 17 characterized by are recording being among the milk of an animal.
[Claim 19] The manufacturing method of this polypeptide which grows the transgenic plant which holds a recombinant DNA according to claim 7, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into this vegetation, and is characterized by extracting this polypeptide from the inside of this vegetation.
[Claim 20] The manufacturing method of this polypeptide characterized by compounding the polypeptide in which this DNA carries out a code by imprint / translation system in in vitro using DNA given in any 1 term of claims 4-6.
[Claim 21] The antibody which recognizes the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 22] The oligonucleotide or this nucleotide which has the array which consists of five to 60 base by which any 1 term of claims 4-6 was followed in the base sequence of DNA of a publication, and the oligonucleotide which has a complementary array.
[Claim 23] How to detect the manifestation including carrying out hybridization to any 1 term of claims 4-6, using DNA or the oligonucleotide according to claim 22 of a publication as a probe of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 24] How to detect the manifestation including performing polymerase chain reaction using the oligonucleotide according to claim 22 as a primer of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 25] How to detect the variation of DNA which carries out the code of the polypeptide given in any 1 term of claims 1-3 by the hybridization method using DNA or the oligonucleotide according to claim 22 of a publication in any 1 term of claims 4-6.
[Claim 26] How to detect the variation including performing polymerase chain reaction using an oligonucleotide according to claim 22 of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 27] An approach given in any 1 term of claims 23-26 used in order to detect the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, or the disease accompanied by unusual cell proliferation.
[Claim 28] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The approach according to claim 27 the disease accompanied by activation of unusual immunocyte is allergy, atopy, asthma, pollinosis, respiratory tract irritation, or an autoimmune disease, and the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor.
[Claim 29] How to control the imprint of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3 characterized by using DNA or the oligonucleotide according to claim 22 of a publication for any 1 term of claims 4-6, or the translation of mRNA.
[Claim 30] How to acquire the promoterregion and the imprint regulatory region of DNA which

are characterized by using DNA or the oligonucleotide according to claim 22 of a publication for any 1 term of claims 4-6 and which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 31] Physic which contains the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 32] Physic which contains the recombinant vector of a publication in DNA given in any 1 term of claims 4-6, claim 8, or any 1 term of 9.
[Claim 33] Physic containing an antibody according to claim 21.
[Claim 34] Physic containing an oligonucleotide according to claim 22.
[Claim 35] Physic according to claim 31 characterized by a polypeptide having an immunity activation operation.
[Claim 36] Physic according to claim 35 characterized by guiding antitumor activity and antiviral activity through an immunity activation operation.
[Claim 37] Physic given in any 1 term of claims 32-34 whose physic is the physic for the therapy of the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.
[Claim 38] Physic given in any 1 term of claims 32-34 whose physic is the physic for a diagnosis of the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, or the disease accompanied by unusual cell proliferation.
[Claim 39] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, Physic according to claim 37 or 38 whose disease accompanied by unusual cell proliferation it is pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[Claim 40] It is characterized by using the polypeptide of a publication for any 1 term of claims 1-3. The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.
[Claim 41] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent

diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening procedure according to claim 40 whose disease accompanied by unusual cell proliferation it is pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[Claim 42] Physic which acts on a polypeptide given in any 1 term of claims 1-3 acquired by the screening approach according to claim 40 or 41 specifically.
[Claim 43] It is characterized by using the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3 obtained by the approach according to claim 30. The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.
[Claim 44] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening approach according to claim 43 that it is pollinosis, respiratory tract irritation, or an autoimmune disease, the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor, and the disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[Claim 45] Physic which acts on the promoterregion and the imprint regulatory region of DNA which are obtained by the screening approach according to claim 43 or 44, and which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3 specifically.
[Claim 46] The immunological detecting method of a polypeptide given in any 1 term of claims 1-3 characterized by using an antibody according to claim 21.
[Claim 47] The immunity staining method characterized by detecting the polypeptide of a publication in any 1 term of claims 1-3 using an antibody according to claim 21.
[Claim 48] How to screen the matter which controls or promotes the imprint or translation of DNA which is characterized by using an antibody according to claim 21, and which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
[Claim 49] The manifestation of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3 is a part or the knock out nonhuman animal controlled completely.
[Claim 50] The activity which the polypeptide of a publication has in any 1 term of claims 1-3 is a part or the knock out nonhuman animal controlled completely.
[Claim 51] The screening approach of a variant polypeptide characterized by using the

polypeptide of a publication for any 1 term of claims 1-3 of having dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 52] The variant polypeptide which is obtained by the screening approach according to claim 51 and which has dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 53] DNA which carries out the code of the variant polypeptide according to claim 52.
[Claim 54] The screening approach of a variant polypeptide characterized by using the polypeptide of a publication for any 1 term of claims 1-3 of having the variation which raises this activation to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.
[Claim 55] The variant polypeptide which is acquired by the screening approach according to claim 54 and to which the NF-kappa B activation ability of the polypeptide of a publication went up in any 1 term of claims 1-3.
[Claim 56] DNA which carries out the code of the variant polypeptide according to claim 55.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] DNA which carries out the code of a polypeptide with new this invention, and this polypeptide, The transformant which holds the recombinant DNA which includes this DNA in a vector and is obtained, and this recombinant DNA, The manufacturing method of this polypeptide using this transformant, the analysis method of the amount of manifestations of this DNA and variation which used the oligonucleotide obtained from this DNA, The immunity staining method using the antibody and this antibody which recognize this polypeptide, the activity rise alteration object which introduced variation into this polypeptide by deletion, insertion, a permutation, etc., The dominant negative variant which introduced variation into this polypeptide by deletion, insertion, a permutation, etc., The screening procedure of a compound which fluctuates the activity of this polypeptide, the screening procedure of a compound which fluctuates the manifestation of this DNA, It is related with the compound obtained by the screening procedures of a compound which fluctuate the effectiveness of the imprint by the promotor DNA who manages the imprint of this DNA, and this promotor DNA, and these screening procedures, the knock out animal to which this DNA was suffered a loss or mutated.
[0002]
[Description of the Prior Art] nuclear factor-kappaB (following, NF-kappaB) was identified as a transcription factor to be combined with the enhancer in connection with the immunoglobulin light chain (Ig light chain) gene expression in a B cell in 1986 [Cell, 46, 705-716 (1986), Cell, and 47,921-928 (1986)].
[0003] NF-kappa B consists of heterodimers of two or more molecules belonging to a Rel family, and NF-kappa B generally guided in many cells is considered to be the heterodimer of p50 and RelA [Mol.Cell.Biol., 12, and 674-684 (1992)]. Existence of the factor IkappaB which controls NF-kappa B has also become clear. IkappaB By forming NF-kappa B and complex at the time of no stimulating, and carrying out the mask of the nuclear shift signal of NF-kappa B [Science which has controlled nuclear shift, 242, and 540-546 (1988), Cell, 65, 1281-1289 (1991), Cell, 68, and 1109-1120 (1992), EMBO J., 12, 3893-3901 (1993), Cell, 78, 773-785 (1994), Cell, 87, and 13-20 (1996) --] . the signal transfer molecule which IkappaB will mention later if a cell is stimulated by a tumor necrosis factor alpha (following, TNF-alpha) etc. -- 32 and the 36th serine -- phosphorylation -- it continues, and it is ubiquitin-ized and is decomposed by proteasome. If IkappaB is decomposed, the shift to a nucleus of NF-kappa B will be attained, and it will come to guide various gene expression with an enhancer [Cell, 80, 529-532 (1995), Cell, 80, and 57 3-582 (1995)].
[0004] As the matter which activates NF-kappa B, or a stimulus, cytokine [TNF-alpha, A tumor necrosis factor beta (following, TNF-beta), interleukin 1 alpha (Following and IL-1alpha), interleukin 1 beta (following and IL-1beta)], such as interleukin 2 (the following, IL-2) and a leukemia inhibitor (following, LIF), T cell mitogen (an antigen stimulus, lectin, and an anti-T cell receptor antibody --) Anti-CD2 antibody, anti-CD3 antibody, anti-CD28 antibody, calcium ionophore, B cell mitogen (an anti-IgM antibody, anti-CD40), leukotriene, Lipopolysaccharide (following, LPS), phorbol myristate acetate (Following, PMA), parasitism somesthesis stain, and

virus infection [human immunodeficiency virus (The following, HIV-1), a human T cell leukemia virus 1 (the following, HTLV-1), A hepatitis B virus (following, HBV), an Epatein-Barr virus (The following, EBV), a cytomegalovirus (following, CMV), a herpes simplex virus 1 (The following, HSV-1), a human herpesvirus 6 (the following, HHV-6),], such as Newcastle disease virus (following, NDV), Sendai Virus, and adenovirus, A virus product (double stranded RNA, Tax and HBX, EBNA-2, LMP-1 grade), DNA destructive matter and protein synthesis inhibitor (for example, cycloheximide) Ultraviolet rays, a radiation, oxidation stress, etc. are known [Biochemica et Biophysica Acta, 1072, 63-80 (1991), Annu.Rev.Cell Biol.10, and 405-455 (1994)].

[0005] moreover, as a molecule in which an induction manifestation is carried out by activation of NF-kappa B (1) To an inflammatory response and an immune response at control of a **** molecule group and (2) apotosis **** molecule group, (3) The **** molecule group, the molecule group about (4) viruses, etc. are known by generating and differentiation. [Biochemica et Biophysica Acta, 1072, and 63-80 (199 1), Annu.Rev.Cell Biol.10, 405-455 (1994)], and an induction manifestation are various.
[0006] As a molecule by which an induction manifestation is carried out, specifically Cytokine [IL-1alpha, IL-1beta, IL-2, interleukin 3 (the following, IL-3), interleukin 6 (The following, IL-6), interleukin 8 (the following, IL-8), interleukin 12 (The following, IL-12), TNF-alpha, TNF-beta, interferon beta], a cell growth factor [macrophage colony-stimulating factor (The following, IFN-beta) (The following, M-CSF), a granulocyte macrophage colony-stimulating factor (Following and GM-CSF), granulocyte colony-stimulating factor (following, G-CSF)], A receptor [interleukin 1 receptor (following and IL-1R) antagonist, The interleukin 2 receptor alpha (following and IL-2Ralpha), an immunoglobulin kappa light chain (The following, Ig-kappa-LC), T-cell receptorbeta, a major histocompatibility antigen Classes I and II, beta 2-microglobulin], adhesion factor [endothelialleucocyte adhesionmolecule-1 (The following, MHC) (The following, ELAM-1), vascula r cell adhesionmolecule -1 (Following and VCAM-1) intercellularadhesion molecule-1 (The following, ICAM-1)] and acute stage protein (blood serum amyloid A precursive protein --) Angiotensinogen, the complement factor B, the complement factor C3, the complement factor C4, An induction type NO synthase (following, iNOS), cyclooxygenase 2 (The following, COX-2), a vascular endothelial cell growth factor acceptor (following, VEGF-R2), Transcription factor [c-Rel, p105, I kappa-alpha, c-Myc, an interferon regulator], vimentin, virus [HIV-1, HIV-2, a rhesus monkey immunodeficiency disease virus (The following, IRF-1) (The following, SIVmac), CMV, HSV-1, the rhesus monkey virus 40 (following, SV40), adenovirus], etc. are known [a protein nucleic-acid enzyme, 41, and 1198-1209 (1996)].
[0007] As for the signal transfer about NF-kappa B activation, the elucidation is progressing about TNF-alpha and IL-1. In the activation signal from TNF-alpha A TNF receptor (TNFR1 or TNFR2), TNF receptor-associate d death domain protein (The following, TRADD), TNFR-associated factor -2 (The following, TRAF2), receptor interacting protein (The following, RIP), NF-kappa B-inducing kinase (The following, NIK), IkappaB kinase (following, IKK) [IKKalpha, IKKbeta, IKKgamma (NEMO)], IKK-co mplex-associated protein (following, IKAP), etc. are found out as an activation molecule. [EMBO J., 14, and 2876-288 3 (1995), Science, 267, and 1485-1489 (1995), GENES & DEVELOPMENT, 9, 1586-1597 (1995), Cell, 84, 853-862 (1996), Nature, 388, and 548-554 (1997), Cell, 90,373-383 (1997), Science, 278, and 860-866 (1 997), Science, 278, 866-869 (1997), Cell, 91, 243-252 (1997), Nature, 395, and 292-296 (1998) --] .
[0008] In the activation signal from IL-1 IL-1 recptor 1 (Following and IL-1RI) IL-1 receptor accessory protein (Following and IL-1RAcP), Myd88, IL-1 receptor-associated kinase TNF receptor-associated factor 6 (The following, IRAK) (The following, TRAF6), and TAK1 binding protein 1 [Science by which (the following, TAB1), Transforming gro wth factor-beta-activated kinase 1 (TAK1), etc. are found out as an activation molecule, 270, and 2008-2011 (1995), Nature, 398, 252-256 (1999)].
[0009] It has been thought that the enzyme (NF-kappa B kinase) which phosphorizes NF-kappa B is concerned with enhancement of a NF-kappa B signal on the other hand [J.Biol.Chem.268, 26790-26795 (1993), EMBO J.13, and 4597-4607 (1994)]. As mentioned above, although it is known that very many molecules are participating in activation of NF-kappa B, all the role of the

identified molecules is not solved. In the stimulus of those other than TNF-alpha, such as ultraviolet rays and oxidation stress, or IL-1, the actual condition is that most molecules in connection with activation of NF-kappa B are not solved. furthermore -- even if it sees the tissue specific expression of a Rel family molecule -- an organization -- [Science, 284, 313-316 (1999), Science, 284, 316-320 (1999), Science, 284, 321-325 (1999), Immunity, 10, 421-429 (1999), Nature Genet, 22, and 74-77] the activation device of specific NF-kappa B is expected to be (1999).

[0010] As mentioned above, it is very useful it to be thought for that many [still] strange molecules in the living body concerned with activation of NF-kappa B exist, and to discover and use these genes for the therapy of the disease in which an elucidation or NF-kappa B of symptoms participates. NF-kappa B is bearing the very important role in rise of an immune response in the living body so that the molecule group which carries out an induction manifestation by activation of the molecule group which activates NF-kappa B mentioned above, or NF-kappa B may also show. The cytokine of TNF-alpha which has antitumor or antiviral activity, or IL-1 grade demonstrates a part for the principal part of the operation through activation of NF-kappa B. Moreover, the cytokine which carries out an induction manifestation by NF-kappa B, such as IL-1, IL-2, IL-12, TNF-alpha, and IFN-beta, also rises the immunoreaction in a living body or an organization, and has antitumor or antiviral activity.

[0011] Thus, it is a well-known fact that activation of NF-kappa B controls a neoplasm and a virus in an actual disease, and it is thought that the thing of in the living body or a living body for which the activity of NF-kappa B is artificially raised in an organization in part is very effective in rise of an immune response or enhancement of antitumor and antiviral activity. Therefore, discovery and acquisition of a NF-kappa B activation rise variant are still very more useful in discovery of DNA which carries out the code of the polypeptide and it which activate NF-kappa B and acquisition, and the physic that used antitumor and antivirotic one as the target.

[0012] On the other hand, cytokine, such as IL-1 which carries out an induction manifestation by NF-kappa B, IL-6, IL-8, and TNF-alpha, is also called inflammatory cytokine, and the immune response which rose too much by these cytokine causes various diseases. These cytokine activates a macrophage, neutrophil leucocyte, a lymphocyte, etc., and works towards exacerbation in an inflammatory tissue. Moreover, the adhesion molecules of ELAM-1, VCAM-1, and ICAM-1 grade guided by NF-kappa B [Mol.Cell.Biol. which promotes infiltration in the organization of a leucocyte and rises accumulation of the leucocyte in an inflammatory tissue, 14, and 5701 (1994), Mol.Cell.Biol., 14, 5820 (1994), Pro.Natl.Acad.Sci USA, 90, and 3943 (1993) --] . The enzyme of iNOS or COX-2 grade produces a nitrogen monoxide (following, NO) and prostaglandin E2, respectively, and acts on the escape of acute inflammation or a blood vessel.

[0013] That is, it is thought that NF-kappa B is bearing the central role in acute inflammation and the chronic inflammation through these cells or molecules. Activation of NF-kappa B is actually reported by the synovial membrane of rheumatoid arthritis, the intestinal tract of Crohn's disease, and asthmatic lung tissue. Therefore, in the disease at large in which inflammation, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, chronic hepatitis B, chronic hepatitis C, graft versus host disease, an insulin dependency and non-dependency diabetes mellitus, traumatic brain injury, inflammatory bowel disease, septicemia, and microorganism infection, participates, NF-kappa B is the important target of a symptoms elucidation and remedy development.

[0014] In connection with cancer, EBV is considered for a Burkitt lymphoma (Burkitt lymphoma), the Hodgkin (Hodgkin) disease, T and B, a spontaneous killer cell lymphoma, EBV related gastric cancer, etc. as a cause. TRADD, TRAF, and association are possible for latent membrane protein (the following, LMP1) in which especially EBV carries out a code, a host's NF-kappa B is activated, and it is thought that it is participating in immortalization [EMBO J., 16, 6478-6485 (1997), J.Virology, 69, 2168-2174 (1995), Oncogene, 18, 7161-7167 (1999), Gene Th erapy, and 5,905-912 (1998)]. Moreover, adult T-cell leukemia (adult T-cell leukemia: ATL) Tax infection by HTLV-1 is the cause and especially HTLV-1 carries out [Tax] a code NF-kappa B is activated through association to IkappaB, or activation of IKK. It is thought that apotosis is checked [J.Biol.Chem., 273, 15891-15894 (1999), J.Biol.Chem., 274, and 34417-34424 (1999)]. The various

adhesion molecules which NF-kappa B guides are participating in transition of a cancer cell, and the vascularization through the apotosis inhibition activity and VEGF-R2 by NF-kappa B is participating in growth of a cancer cell. As mentioned above, NF-kappa B is an important innovative drug development or a therapy target also in the field of cancer.

[0015] Furthermore, also in the viral disease which contains NF-kappa B other than cancers, such as an acquired immunode-ficiency syndrome, as a transcription factor, NF-kappa B is an important innovative drug development or a therapy target. Moreover, there is a report called a cause and control of the cellular infiltration also according [ischemia re-reflux failures, such as ischemic encephalopathy,] to NF-kappa B activation and apotosis etc. is considered that NF-kappa B has played the important role in the onset of the disease accompanied by unusual differentiation growth of a smooth muscle cell including arteriosclerosis, the restenosis, etc.

[0016] Although it has been shown clearly that it is what the anti-inflammatory activity of a steroid, the anti-inflammatory activity of aspirin, etc. depend on inhibition of NF-kappa B, there are no drugs screened as what checks specifically [Sceience, 270, 283-286 (1995), Sceience, 270, 286-290 (1995), Molecular and Cellular Biology, 15 and 943-953 (1995)] and NF-kappa B in recent years. It also has many troubles that the drugs known as a thing in connection with inhibition of the existing NF-kappa B have that a side effect is strong, and low selectivity and singularity etc., and compound retrieval to which NF-kappa B was targeted for the purpose of development of a powerful and new antiinflammatory drug with few side effects is performed. As mentioned above, the new polypeptide which activates NF-kappa B is useful on industry, and acquisition of DNA which carries out the code of these polypeptides and it has been called for.

[0017]

[Problem(s) to be Solved by the Invention] This invention Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, traumatic brain injury, The disease accompanied by infection and inflammation of inflammatory bowel disease etc., a Burkitt lymphoma, Hodgkin's disease, The disease accompanied by unusual cell proliferations, such as various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as articular rheumatism and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic infla mmatory response syndrome), Remedies, such as adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome), DNA which carries out the code of a useful polypeptide and this polypeptide to retrieval of a prophylactic and a diagnostic drug and development, It is in offering the antibody which recognizes the gene therapy using the antisense DNA/RNA of this DNA, and this DNA, and this polypeptide, the activity rise alteration object of this polypeptide, the dominant negative variants of this polypeptide, and these directions.

[0018]

[Means for Solving the Problem] As a result of inquiring wholeheartedly in order to solve the above-mentioned technical problem, this invention persons succeed in acquiring DNA which carries out the code of the factor to which activation of NF-kappa B including a new amino acid sequence is urged, and this factor, and came to complete this invention. That is, this invention relates to the following (1) - (54).

[0019] (1) The polypeptide which has the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5.

(2) The polypeptide which has the activity which one or more amino acid consists [activity] of deletion and amino acid sequences permuted and/or added in the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array

numbers 1-5, and raises the activity of NF-kappa B.
[0020] (3) The polypeptide which has the activity which raises the activity of NF-kappa B, including the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and the amino acid sequence which has 60% or more of homology.
(4) (1) DNA which carries out the code of the polypeptide of a publication to any 1 term of - (3).
(5) DNA which has the base sequence expressed with either of the array numbers 6-10.
[0021] (6) DNA which carries out the code of the polypeptide which has the activity which it is [activity] DNA given in (4) or (5), and DNA hybridized under stringent conditions, and raises the activity of transcription factor NF-kappa B.
(7) (4) Recombinant vector which includes DNA of a publication in any 1 term of - (6) at a vector, and is obtained.
(8) (4) Recombinant vector which includes in a vector RNA which becomes any 1 term of - (6) from DNA of a publication, and a homologous array, and is obtained.
[0022] (9) The recombinant vector given in (8) given RNA is a single strand.
(10) The transformant which holds a recombinant vector given in (7).
(11) The transformant given in (10) a given transformant is a transformant chosen from the group which consists of a microorganism, an animal cell, a plant cell, and an insect cell.
(12) The transformant given in (11) a given microorganism is a microorganism belonging to an Escherichia group.
[0023] (13) an animal cell -- a mouse - myeloma -- a cell -- a rat - myeloma -- a cell -- a mouse - a hybridoma -- a cell -- CHO -- a cell -- BHK -- a cell -- an African green monkey - - the kidney -- a cell -- Namalwa -- a cell -- Namalwa KJM - one -- a cell -- Homo sapiens - - an embryo -- the kidney -- a cell -- and -- Homo sapiens -- a leukemic cell -- from -- choosing -- having -- an animal cell -- it is -- (-- 11 --) -- a publication -- a transformant .
(14) The transformant given in (11) a given insect cell is an insect cell chosen from the ovarian cell of Spodoptera frugiperda, the ovarian cell of Trichoplusia ni, and the ovarian cell of a silkworm.
[0024] (15) The transformant given in (10) a given transformant is a nonhuman transgenic animal or a transgenic plant.
(16) (10) The manufacture approach of this polypeptide which cultivates a transformant given in any 1 term of - (14) to a culture medium, is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into a culture, and is characterized by extracting this polypeptide from this culture.
[0025] (17) The manufacture approach of this polypeptide which breeds the nonhuman transgenic animal which holds a recombinant DNA given in (7), is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into this animal, and is characterized by extracting this polypeptide from the inside of this animal.
(18) The manufacturing method given in (17) characterized by are recording being among the milk of an animal.
[0026] (19) The manufacturing method of this polypeptide which grows the transgenic plant which holds a recombinant DNA given in (7), is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into this vegetation, and is characterized by extracting this polypeptide from the inside of this vegetation.
(20) (4) Manufacturing method of this polypeptide characterized by compounding the polypeptide in which this DNA carries out a code by imprint / translation system in in vitro using DNA given in any 1 term of - (6).
[0027] (21) (1) Antibody which recognizes the polypeptide of a publication in any 1 term of - (3).
(22) (4) The oligonucleotide or this nucleotide which has the array which consists of 5 by which any 1 term of - (6) was followed in the base sequence of DNA of a publication - 60 base, and oligonucleotide which has a complementary array.
(23) How to detect the manifestation including carrying out hybridization to any 1 term of - (6), using an oligonucleotide DNA of a publication, or given in (4) (22) as a probe of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3).

[0028] (24) How to detect the manifestation including performing polymerase chain reaction using the oligonucleotide given in (22) as a primer of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3).
(25) How to detect the variation of DNA which carries out the code of the polypeptide given in any 1 term of (1) - (3) by the hybridization method using an oligonucleotide DNA of a publication, or given in (4) (22) in any 1 term of - (6).
[0029] (26) How to detect the variation of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3) which includes performing polymerase chain reaction using an oligonucleotide given in (22).
(27) infection -- inflammation -- following -- a disease -- being unusual -- a smooth muscle cell -- differentiation -- growth -- following -- a disease -- being unusual -- fibroblast -- activation -- following -- a disease -- being unusual -- a synovial membrane -- an organization -- activation -- following -- a disease -- the pancreas -- a beta cell -- a failure -- following -- a disease -- being unusual -- an osteoclast -- activation -- following -- a disease -- being unusual -- immunocyte -- activation -- following -- a disease -- or -- being unusual -- cell proliferation -- following -- a disease -- detecting -- a sake -- using -- (-- 23 --) - (-- 26 --) -- some -- one -- a term -- a publication -- an approach .
[0030] (28) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The approach given in (27) the disease accompanied by activation of unusual immunocyte is allergy, atopy, asthma, pollinosis, respiratory tract irritation, or an autoimmune disease, and the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor.
[0031] (29) How to control the imprint of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3) characterized by using an oligonucleotide DNA of a publication, or given in (4) (22) for any 1 term of - (6), or the translation of mRNA.
(30) How to acquire the promoterregion and the imprint regulatory region of DNA which are characterized by using an oligonucleotide DNA of a publication, or given in (4) (22) for any 1 term of - (6) and which carry out the code of the polypeptide of a publication to any 1 term of (1) - (3).
[0032] (31) (1) Physic which contains the polypeptide of a publication in any 1 term of - (3).
(32) (4) Physic which contains the recombinant vector of a publication in any 1 term of DNA given in any 1 term of - (6), (8), or (9).
(33) Physic containing an antibody given in (21).
(34) Physic containing an oligonucleotide given in (22).
[0033] (35) Physic given in (31) characterized by a polypeptide having an immunity activation operation.
(36) Physic given in (35) characterized by guiding antitumor activity and antiviral activity through an immunity activation operation.
(37) The disease accompanied by infection or inflammation in physic, the disease accompanied by differentiation growth of an unusual smooth muscle cell, The disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, The disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, being unusual -- immunocyte -- activation -- following -- a disease -- being unusual -- cell proliferation -- following -- a disease -- or -- a nerve cell -- a failure -- being based -- a disease -- a therapy -- and/or -- prevention -- a

sake -- physic -- it is -- (-- 32 --) - (-- 34 --) -- some -- one -- a term -- a publication -- physic .
[0034] (38) physic -- infection -- inflammation -- following -- a disease -- being unusual -- a smooth muscle cell -- differentiation -- growth -- following -- a disease -- being unusual -- fibroblast -- activation -- following -- a disease -- being unusual -- a synovial membrane -- an organization -- activation -- following -- a disease -- the pancreas -- a cell -- a failure -- following -- a disease -- being unusual -- an osteoclast -- activation -- following -- a disease -- being unusual -- immunocyte -- activation -- following -- a disease -- or -- being unusual -- cell proliferation -- following -- a disease -- a diagnosis -- a sake -- physic -- it is -- (-- 32 --) - -- (-- 34 --) -- some -- one -- a term -- a publication -- physic .
[0035] (39) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, Physic of (37) or (38) publications whose disease accompanied by unusual cell proliferation are pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[0036] (40) (1) It is characterized by using the polypeptide of a publication for any 1 term of - (3). The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.
[0037] (41) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening procedure given [are pollinosis, respiratory tract irritation, or an autoimmune disease, and the given disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor] in (40) the given disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[0038] (42) Physic which acts on a polypeptide given in any 1 term of (1) - (3) obtained by the screening approach (40) or given in (41) specifically.
(43) It is characterized by using the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of a publication to any 1 term of (1) - (3) obtained by the approach given in (30). The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease

accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.
[0039] (44) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening approach given in (43) that it is pollinosis, respiratory tract irritation, or an autoimmune disease, the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor, and the disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.
[0040] (45) Physic which acts on the promoterregion and the imprint regulatory region of DNA which are obtained by the screening approach (43) or given in (44), and which carry out the code of the polypeptide of a publication to any 1 term of (1) - (3) specifically.
(46) The immunological detecting method of a polypeptide given in any 1 term of (1) - (3) characterized by using an antibody given in (21).
(47) The immunity staining method characterized by detecting the polypeptide of a publication in any 1 term of (1) - (3) using an antibody given in (21).
[0041] (48) How to screen the matter which controls or promotes the imprint or translation of DNA which is characterized by using an antibody given in (21), and which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3).
(49) (1) The manifestation of DNA which carries out the code of the polypeptide of a publication to any 1 term of - (3) is a part or the knock out nonhuman animal controlled completely.
(50) (1) The activity which the polypeptide of a publication has in any 1 term of - (3) is a part or the knock out nonhuman animal controlled completely.
[0042] (51) The screening approach of a variant polypeptide of having dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of (1)(1) characterized by using polypeptide of publication for any 1 term of - (3) - (3).
(52) the variant polypeptide which has dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of acquisition **** and (1) - (3) by the screening approach given in (51).
(53) DNA which carries out the code of the variant polypeptide given in (52).
[0043] (54) The screening approach of a variant polypeptide of having the variation which is characterized by using the polypeptide of a publication for any 1 term of - (3) and which raises this activation to NF-kappa B activation of the polypeptide of a publication in any 1 term of (1) (1) - (3).
(55) The variant polypeptide which is acquired by the screening approach given in (54) and to which the NF-kappa B activation ability of the polypeptide of a publication went up in any 1 term of (1) - (3).
(56) DNA which carries out the code of the variant polypeptide given in (55).
[0044]
[Embodiment of the Invention] In the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with the polypeptide 2. array numbers 1-5 which have the amino acid sequence chosen from the group which consists of an amino acid sequence

expressed with either of 1. array numbers 1-5 as a polypeptide of this invention one or more amino acid Deletion, The amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the polypeptide 3. array numbers 1-5 which has the activity which it consists [activity] of an amino acid sequence permuted and/or added, and raises the activity of NF-kappa B, and the amino acid sequence which has 60% or more of homology are included. And the polypeptide which has the activity which raises the activity of NF-kappa B can be mentioned.

[0045] The polypeptide which has the amino acid sequence to which one or more amino acid was ****(ed), permuted and/or added in the polypeptide which has the above-mentioned amino acid sequence Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989 (It abbreviates to the 2nd edition of molecular cloning hereafter), Current Protocols in Molecular Biology, John Wiley & Sons, 1987-1997 (It abbreviates to current PUROTO call Inn molecular biology hereafter) Nucleic Acids Research, 10, and 6487 (1982), Proc.Natl.Acad.Sci., USA, 79, and 6409 (1982), Gene, 34, 315 (1985), Nucleic Acids Research, 13, 4431 (1985), The site-specific mutation introducing method of a publication is used for Proc.Natl.Acad.Sc i USA, 82, 488 (1985), etc. For example, it can carry out by introducing site-specific mutation into DNA which carries out the code of the polypeptide which has one amino acid sequence of the array numbers 1-5. although the number of deletion and the amino acid permuted and/or added comes out of 1 partly, and there is and especially the number is not limited -- the technique of common knowledge, such as the above-mentioned site-specific mutation introducing method, -- the number of deletion and extent which can be permuted or added -- it is -- for example, 1- dozens of pieces are 1-5 pieces still more preferably 1-10 pieces more preferably 1-20 pieces.

[0046] Moreover, as a polypeptide of this invention, the amino acid sequence of a publication and the amino acid sequence which has 60% or more of homology are included in either of the array numbers 1-5. The homology with an amino acid sequence given in either of the array numbers 1-5 With analysis software, such as BLAST [J.Mol.Biol., 215, and 403 (1990)] and FASTA (Methods in Enzymology, 183, 63-69) It is most preferably [97% or more of] more preferably desirable [70% or more / 80% or more] at least 60% or more, when it calculates using a default (initialization) parameter 95% or more especially preferably 90% or more still more preferably preferably.

[0047] DNA which has the base sequence expressed with either of the DNA3. array numbers 6-10 which are DNA of the DNA2. claim 4 publication which carries out the code of the polypeptide of a publication to any 1 term of 1. claims 1-3 as DNA of this invention, and DNA hybridized under stringent conditions, and carry out the code of the polypeptide which has the activity which raises the activity of transcription factor NF-kappa B can be mentioned.

[0048] Since two or more sorts of gene codes generally exist per amino acid, it is contained in DNA of this invention, if the code of the polypeptide of this invention is carried out even if either of the array numbers 6-10 is DNA which has a different base sequence. With DNA hybridized under stringent conditions For example, DNA of this inventions, such as DNA which has the base sequence expressed with the array numbers 6, 7, 8, 9, or 10, or some of its fragments are used as a probe. DNA obtained by using a colony hybridization method, a plaque hybridization method, or a Southern blotting hybridization method is meant. The filter which fixed DNA of a colony or the plaque origin is specifically used. The SSC solution of 0.1 - 2 double concentration the bottom of the sodium chloride existence of 0.7 - 1.0 mol/l, and after performing hybridization at 65 degrees C (the SSC solution of concentration 1 time) DNA which can be identified by washing a filter under 65-degree-C conditions can be mentioned using a 150 mmol/l sodium chloride and 15 mmol/l sodium-citrate twist. Hybridization is the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, and D NACloning 1. : It can carry out according to the approach indicated by Core Techniques, A Practical Approach, Second Edition, Oxford University, and 1995 grades.

[0049] As DNA which can be hybridized, specifically When it calculates with analysis software, such as BLAST and FASTA, using a default (initialization) parameter The base sequence expressed with the array numbers 6, 7, 8, 9, or 10, and DNA which has at least 60% or more of

homology, DNA which has 98% or more of homology most preferably can be mentioned especially 95% or more preferably 90% or more still more preferably 80% or more 70% or more.

[0050] Hereafter, this invention is explained to a detail.

1. Preparation Homo sapiens mRNA of DNA of this invention may use a commercial thing (for example, product made from Clontech), and may prepare from human tissue as the following. as the approach of preparing all RNA from an organization -- thiocyanic acid guanidine - trifluoroacetic acid caesium method [Methods in Enzymology, 154, and 3] (19 87) acidity thiocyanic acid guanidine phenol chloroform (AGPC) -- law [Analytical Biochemistry, 162, 156 (1987), the experimental medicine, 9, and 1937 (1991)] etc. is mentioned. Moreover, as an approach of preparing mRNA as polyA+RNA from all RNA, the oligo (dT) fixed cellulose column method (the 2nd edition of molecular cloning) etc. is mentioned. Furthermore, FastTrack mRNA Isolation Kit (product made from Invitrogen), Quick Prep mRNA mRNA can be prepared by using kits, such as Purification Kit (product made from Pharmacia).

[0051] A cDNA library is produced from prepared human tissue mRNA. As a cDNA library producing method, the 2nd edition of molecular cloning, Current PUROTO call Inn molecular biology, A Laboratory Manual, 2 nd Ed., the approach indicated by 1989 grades, (Or a commercial kit, for example, SuperScript Plasmid System for cDNA, Synthesis and Plasmid Cloning (product made from Life Technologies)) The approach using ZAP-cDNA Synthesis Kit (product made from STRATAGENE) etc. is mentioned.

[0052] As a cloning vector for producing a cDNA library, if independence reproduction can be carried out in Escherichia coli K-12, a phage vector, a plasmid vector, etc. can use either. Specifically The product made from ZAP Express[STRATAGENE, Strategies, 5, 58 (1992),], and pBluescript II SK -- (+ [Nucleic Acids Research, 17, and 9494 (1989)]) -- Lambda ZAP II (product made from STRATAGENE), lambdagt10, and lambdagt11 [DNA cloni ng, A Practical Approach, 1, and 49 (1985)], lambdaTriplEx (product made from Clontech), lambdaExCell (product made from Pharmacia), pT7T318U (product made from Pharmacia), pcD2[Mol.Cell.Biol., 3, 280 (1983)], pUC18 [Gene, 33, and 103 (1985)], etc. can be mentioned.

[0053] Either can be used if it is a microorganism belonging to Escherichia coli as a host microorganism. Specifically The product made from Escherichia coli XL1-Blue MRF'[STRATAGENE, Strategies, 5, 81 (1992)], and Escherichia coli C600 [Genetics, 39, and 440 (1954)], Esherichia coli Y1088 [Science, 222, and 778 (1983)], Escherichiacoli Y1090 [Science, 222, and 778 (1983)], Escherichia coli NM522 [J.Mol.Biol., 166, and 1 (1983)], Eshe richia coli K802 [J.Mol.Biol., 16, and 118 (1966)], Escherichia coli JM105 [Gene, 38, and 275 (1985)], etc. are used.

[0054] Although this cDNA library may be used for the following analyses as it is, in order to lower the rate of the imperfect length cDNA and to acquire the perfect length cDNA efficiently if possible Oligo-capping method [Gene which Sugano and others developed, 138, and 171 (1994), Gene, 200, 149 (1997), a protein nucleic-acid enzyme, 41, and 603 (1996), The experimental medicine, 11, 2491 (1993), and cDNA cloning, Yodosha (1996) Method of producing a gene library, Yodosha (1994) The cDNA library prepared using] may be used for the following analyses.

[0055] The base sequence of this DNA is determined by isolating each clone from the produced cDNA library, and analyzing the base sequence of cDNA from an end using base sequence analysis apparatus, such as the base sequence analysis approach usually used, for example, the dideoxy chain termination method of Sanger and others (Sanger), [Proc.Natl.Acad.Sci.USA, 74, 54 63 (1977)], and ABI PRISM377 DNA sequencer (product made from PEBiosystem), about each clone. By translating the acquired base sequence into an amino acid sequence, the amino acid sequence of the polypeptide in which this DNA carries out a code can be acquired.

[0056] Moreover, the base sequence from which the acquired base sequence was acquired [whether it is a new base sequence and], and a base sequence with homology can be searched by comparing the acquired base sequence using homology analyzers, such as a base sequence in base sequence databases, such as GenBank and EMBL, BLAST, and FASTA. Moreover, the family protein suddenly presumed also in the polypeptide in which the base sequence carries out a code and a polypeptide with homology, for example, the polypeptide originating in the corresponding gene in living thing kind with an another rat, the same activity, and the same

function can be searched by comparing the amino acid sequence acquired from the base sequence with amino acid sequence databases, such as SwissProt, PIR, and GenPept.
[0057] Based on the base sequence of the homologous gene which became clear by database retrieval, a specific primer is designed in this gene and PCR is performed by using as mold the single strand cDNA acquired as mentioned above or a cDNA library. When a magnification fragment is obtained, subcloning of this fragment is carried out to a suitable plasmid. subcloning -- a magnification fragment -- as it is -- or a restriction enzyme and DNA polymerase -- after processing and a law -- it can carry out by including in a vector by the method. As a vector, pBluescript SK (-), (the product made from Stratagene), pBluescript II SK (+), (the product made from Stratagene), pDIRECT [Nucreic Acid s Research, 18, and 6069 (1990)], pCR-Script Amp SK (+), (the product made from Stratagene), pT7Blue (product made from Novagen), pCRII (product made from Invitrogen), pCR-TRAP (product made from Genehunter), pNo TAT7 (5'->3' company make), etc. can be mentioned.
[0058] After DNA which consists of one base sequence of the array numbers 6-10 is once acquired and the base sequence is determined, DNA of this invention is acquirable by preparing the primer based on the base sequence of 5 'edge and 3' edge of this base sequence, and amplifying DNA using cDNA or the cDNA library compounded from mRNA contained in the tissue or the cell of Homo sapiens or a nonhuman animal.
[0059] Moreover, DNA of this invention is acquirable by performing colony hybridization and plaque hybridization (the 2nd edition of molecular cloning) to cDNA or the cDNA library compounded from mRNA contained in the tissue or the cell of Homo sapiens or a nonhuman animal by using as a probe an overall length or a part of DNA which consists of one base sequence of the array numbers 6-10.
[0060] DNA of this invention is also acquirable by carrying out chemosynthesis based on the base sequence of determined DNA with DNA synthesis machines, such as a DNA synthesis machine (model 392) of Perkin-Elmer using a HOSUFO aminodite method. As an oligonucleotide of this invention, the derivative (henceforth, derivative oligonucleotide) of oligonucleotides, such as Oligo DNA and Oligo RNA, and this oligonucleotide etc. is mentioned.
[0061] as this oligonucleotide or this oligonucleotide, and the oligonucleotide (henceforth, antisense oligonucleotide) equivalent to a complementary array — for example, in some base sequences of mRNA to detect, the sense primer equivalent to the base sequence by the side of a five prime end, the antisense primer equivalent to the base sequence by the side of a three-dash terminal, etc. can be mentioned. However, the base which is equivalent to a uracil in mRNA serves as thymidine in an oligonucleotide primer.
[0062] As a sense primer and an antisense primer, it is the oligonucleotide which does not change extremely both melting out temperature (Tm) and number of bases, and the thing of the number of 10 - 50 bases is mentioned preferably five to 60 base. What was exchanged for HOSUFORO thioate association in the phosphodiester bond in an oligonucleotide as a derivative oligonucleotide, That from which the phosphodiester bond in an oligonucleotide was changed into N3'-P5' HOSUFO friend date association, That from which RIPOSU and the phosphodiester bond in an oligonucleotide were changed into peptide nucleic-acid association, That by which the uracil in an oligonucleotide was permuted by the C-5 propynyl uracil, That by which the uracil in an oligonucleotide was permuted by the C-5 thiazole uracil, That by which the cytosine in an oligonucleotide was permuted with the C-5 propynyl cytosine, That by which the cytosine in an oligonucleotide was permuted with the phenoxazine qualification cytosine (phenoxazine-modified cytosine), That by which the ribose in an oligonucleotide was permuted by the 2'-methoxyethoxy ribose is mentioned [a cell technology, 16, and 1463 (1997)].
[0063] 2. In host cell this invention used for the detecting method (1) activity detection of NF-kappa B activation of DNA of this invention, if it is the cell which can introduce DNA into intracellular as a host cell used in order to detect the activity of DNA, any cells can be used. As this cell, the cell originating in for example, bacteria and Archea, algae, a fungus, vegetation, an animal, etc. is mentioned. Specifically, the cell of the following living thing origin is mentioned.
[0064] Escherichia coli, Bacillus subtilis, etc. are mentioned as bacteria and Archea. The cyanobacterium of a Synechococcus group or a Synechocystis group etc. is mentioned as algae.

As vegetation, tobacco, Arabidopsis, a tomato, a potato, the rapeseed, cotton, soybeans, a rice, or corn is mentioned. Saccharomyces cerevisiae, Aspergillus nigar, etc. are mentioned as a fungus. Mammalian, Arthropoda, etc. are mentioned as an animal.
[0065] As mammalian, Homo sapiens, an ape, a mouse, a rat, a guinea pig, or a mink is mentioned. Specifically as a human cell, the T cell stock Jurkat [the cell strain of number TIB-512 of an American type culture collection (it is hereafter written as ATCC)], the B cell stock Namalwa (ATCC CRL-1432), the uterine cancer cell strain Hela (ATCC CCL-2), the nephrocyte stock 293 [J.Gen.Viol.36 and 59-72 (1977)], etc. can be used. As a cell of mammalians other than Homo sapiens, ape nephrocyte stock COS-1 (ATCC CRL-16 50), Ape nephrocyte stock COS-7 (ATCC CRL-1651), the Chinese hamster ovary cell (Chinease Hamster Ovary) cell strain CHO (ATCC CRL-9096, ATCC CCL-61), Mouse cell strain Ba/F3 (RIKEN Cell Bank RCB0805), The mouse cell strain L929 (RIKEN Cell Bank RCB0081), rat cell strain NRK-49F (ATCC CRL-1570), the mink cell strain MvlLu (ATCC CCL-64), etc. can be used. A silkworm is mentioned as Arthropoda. Specifically, nine shares of Spodoptera frugiperda Sf, 21 shares of Sf(s), etc. can be used. When retrieval of DNA used as the screening target of the protein nature drugs for a therapy or drugs is the purpose, it is desirable to make the cell of mammalian, especially a human cell into a host.
[0066] (2) If it is the approach of introducing a gene into a host cell as an approach of introducing DNA of transgenics method this invention to a host cell into a host cell, it can use by any approaches. For example, the electroporation method (the Yodosha biotechnology manual series 4 and 23), A calcium phosphate method (the Yodosha biotechnology manual series 4 and 13), The DEAE dextran method (the Yodosha biotechnology manual series 4 and 16), The RIPOFE cushion method (the Yodosha biotechnology manual series 4 and 28), A microinjection method (the Yodosha biotechnology manual series 4 and 36), Well-known approaches, such as the adenovirus method (the Yodosha biotechnology manual series 4 and 43) and the vaccinia virus method (Yodosha biotechnology manual series 4 and 59) retrovirus vector method (the Yodosha biotechnology manual series 4 and 74), can be used.
[0067] (3) Since DNA of approach this invention which acquires DNA of this invention can activate NF-kappa B by making it discovered in a cell, it can acquire DNA of this invention by using the approach of detecting activation of NF-kappa B in a cell. The following approaches are mentioned as an approach of detecting activation of NF-kappa B.
[0068] For example, the approach of analyzing association to imprint regulatory region by the gel shifting method (the Yodosha biotechnology manual series 5 and 107) etc., and the method of detecting the phosphorylation of IkappaB and ubiquitin-ization by western blotting (the Yodosha biotechnology manual series 7 and 179) etc. are mentioned as an approach using a cell extract. Furthermore, the approach of detecting using a reporter gene as an approach of detecting efficiently can be mentioned. As a reporter gene, the gene which carries out the code of luciferase, the Homo sapiens placenta alkaline phosphatase, the beta-galactosidase, urokinase, chloramphenicol acetyltransferase, a human growth hormone, various Greenfluorescent protein (following, GFP), etc. can be used. If it is the promotor who is imprinted by NF-kappa B and gets as a promotor who connects with a reporter gene, any promotors can use. For example, the promotor DNA fragment isolated by starting the promoterregion of a gene where the manifestation is controlled by activation of NF-kappa B by restriction enzyme digestion from Chromosome DNA, the promotor DNA fragment obtained by amplifying by the PCR method by using Chromosome DNA as mold, or the synthetic DNA fragment which has this promotor's base sequence is mentioned.
[0069] Specifically IL-1alpha, IL-1beta, IL-2, IL-3, IL-6, IL-8, IL-12, TNF-alpha, TNF-beta, IFN-beta, M-CSF, GM-CSF, G-CSF, L-2Ralpha, Ig-kappa-LC, T-cell receptorbeta, the MHC class 1, beta 2-microglobulin, LAM-1, VCAM-1, ICAM-1, blood serum amyloid A precursive protein, Angiotensinogen, the complement factor B, the complement factor C3, the complement factor C4, iNOS, COX-2, VEGF-R2, c-Rel, p105, IkappaBalpha, Promotors, such as c-Myc, IRF-1, HIV-1, HIV-2, SIVmac, CMV, HSV-1, SV40, and adenovirus, a synthetic promotor with [one or more] those consensus sequences, etc. are mentioned.
[0070] By the detection approach using a reporter gene, after producing the imprint unit which

connected the reporter gene with the above-mentioned promotor, the cell strain which included the imprint unit in the chromosome of a host cell is produced. After introducing into intracellular [this] the unit which discovers DNA of this invention and making DNA of this invention discover, activation of NF-kappa B is detectable by measuring the amount of manifestations of a reporter gene. Or after producing the imprint unit which connected the reporter gene with the above-mentioned promotor, activation of NF-kappa B is detectable by introducing into coincidence two units, this imprint unit and the unit which discovers DNA of this invention, at a host cell, and measuring the amount of manifestations of a reporter gene.

[0071] 3. Using the approach indicated by the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, etc., by the following approaches, it can be made discovered in a host cell and the polypeptide of manufacture this invention of the polypeptide of this invention can manufacture DNA of this invention.

[0072] The DNA fragment of the suitable die length containing the part which carries out the code of this polypeptide if needed based on an overall length cDNA is prepared. A recombination vector is produced by inserting this DNA fragment or an overall length cDNA in the lower stream of a river of the promotor of a suitable expression vector. The transformant which produces the polypeptide of this invention can be obtained by introducing this recombination vector into the host cell which suited this expression vector.

[0073] As a host cell, if bacteria, yeast, an animal cell, an insect cell, a plant cell, etc. can discover the gene made into the purpose, all can use them. As an expression vector, in the above-mentioned host cell, the nest to the inside of a chromosome is possible, and autonomous replication's being possible or the thing containing a promotor is used for the location which can imprint DNA which carries out the code of the polypeptide of this invention.

[0074] When using procaryotes, such as bacteria, as a host cell, while the recombination vector which comes to contain DNA which carries out the code of the polypeptide of this invention can be replicated autonomously in a procaryote, it is desirable that they are a promotor, a ribosome junction sequence, the gene that carries out the code of the polypeptide of this invention, and the vector which consisted of conclusion arrays of an imprint. In addition, the gene which controls a promotor may be contained in the vector.

[0075] As an expression vector, for example pBTrp2 (product made from Boehrin ger Mannheim), pBTac1 (product made from Boehringer Mannheim), pBTac2 (product made from Boehringer Mannheim), pKK 233-2 (product made from Pharmacia), pSE280 (product made from Invitrogen), pGEMEX-1 (product made from Promega), pQE-8 (product made from QIAGEN), pKYP10 (Provisional-Publication-No. 5 8-110600 No.) and pKYP200 [Agricultural.Biological.Chemistry., 48, and 669 (1984)], pLSA1 [Agric.Bil o.Chem., 53, and 277 (1989)], pGEL1 [Proc.Natl.Acad.Sci.USA, 82, and 4306 (1985)], pBluescript II SK (-), (the product made from Stratagene), From pTrS30[Escherichia coli JM109/pTrS30 (FERM BP-5407), preparation], From pTrS32[Escherichia coli JM109/pTrS32 (FERM BP-5408), preparation], It prepares from pGHA2 [Escherichia coli IGHA2 (FER M BP-400). It prepares from JP,60-221091,A] and pGKA2 [Escherichia coli IGKA2 (FERM BP-6798). JP,60-221091,A] and pTerm2 (U.S. Pat. No. 4,686,191 --) U.S. Pat. No. 4,939,094 and U.S. Pat. No. 5,160,735, pSupex, and pUB110, pTP5, pC194 and pEG400 [J.Bacteriol., 172, and 2392 (1990)], As a . expression vector which can mention pGEX (product made from Pharmacia), a pET system (product made from Novagen), etc. It is desirable to use what adjusted between the Shine-Dalgarno (Shine-Dalgarno) arrays and initiation codons which are a ribosome junction sequence in a suitable distance (for example, six to 18 base).

[0076] As a promotor, as long as it can be discovered in a host cell, what kind of thing may be used. For example, the promotor originating in Escherichia coli, phage, etc., such as a trp promotor (Ptrp), a lac promotor, PL promotor, PR promotor, and T7 promotor, and SPO1 promotor, SPO2 promotor, a penP promotor, etc. can be mentioned. Moreover, the promotor by whom the design alteration was artificially done like the promotor (Ptrpx2) who did 2 serials of the Ptrp, a tac promotor, lacT7 promotor, and a letI promotor [Gene, 44, and 29 (1986)] can use.

[0077] The production rate of the polypeptide made into the purpose can be raised by permuting a base so that it may become the optimal codon for a host's manifestation about the base sequence of the part which carries out the code of the polypeptide of this invention. In the

recombination vector of this invention, although the conclusion array of an imprint is not necessarily required for the manifestation of DNA of this invention, it is desirable to arrange the conclusion array of an imprint directly under a structural gene.

[0078] As a host cell, Escherichia, Serratia, Bacillus, Brevibacterium, The microorganism belonging to Corynebacterium, Microbacterium, Pseudomonas, etc., For example, Escheri chia coli XL1-Blue, Escherichia coli XL2-Blue, Escherichia coli DH1, Escherichia coli MC1000, Escherichia coli KY3276, Escherichia coli W1485, and Escherichia coli JM109, Escherichia coliHB101, Escherichia coliNo.49, Escherichia coli W3110 and Escherichia coliNY49, Serr atia ficaria, Serratia fonticola, Serratialiquefaciens, Serratia marcescens, Baci llus subtilis, Bacillus amyloliquefacines, Brevibacterium ammoniagenes, Brevibacterium immariophilum ATCC14068 and Brevibacterium saccharolyticum ATCC14066, Brevibacterium flavum ATCC14067, Brevibacterium lactofermentum ATCC13869, and Corynebacterium glutamicum ATCC1303 2, Microbacterium ammoniaphilum ATCC15354, and Pseudomonasu sp.D-0110 grade can be mentioned.

[0079] All can be used if it is the approach of introducing DNA to the above-mentioned host cell as the introductory approach of a recombination vector. For example, the approach using calcium ion [Proc.Natl. Acad.Sci.USA, 69, and 2110 (1972)], The approach of a publication etc. can be mentioned to the protoplast method (JP,63-248394,A) or Gene, 17, 107 (1982) and Mole cular & General Genetics, 168, and 111 (1979).

[0080] When using yeast as a host cell, YEP13 (ATCC37115), YEp24 (ATCC37051), YCp50 (ATCC37419), pHS19, and pHS15 grade can be mentioned as an expression vector. As a promotor, as long as it can be discovered in a yeast-fungus stock, which thing may be used, for example, they are the promotor of the gene of glycolytic pathways, such as a hexose kinase, PHO5 promotor, a PGK promotor, a GAP promotor, an ADH promotor, gal1 promotor, gal10 promotor, a heat shock protein promotor, and MF1. A promotor, CUP1 promotor, etc. can be mentioned.

[0081] As a host cell, the microorganism belonging to a Saccharomyces, a clew IBERO married-woman group, the Trichosporon, a SHUWANIO married-woman group, etc., for example, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyce s lactis, Trichosporon pullulans, Schwanniomyces alluvius, etc. can be mentioned. All can be used if it is the approach of introducing DNA into yeast as the introductory approach of a recombination vector. For example, the electroporation method [Methods.Enzymol., 194, and 182 (1990)], The spheroplast method [Proc.Natl.Acad.Sci.USA, 84, and 1929 (1978)], The acetic-acid lithium method [J.Bacteriolog y, 153, and 163 (1983)], an approach given in [Proc.Natl.Acad.Sci.USA, 75, and 1929 (1978)], etc. can be mentioned.

[0082] In using an animal cell as a host, as an expression vector For example, pcDNAI, pcDM8 (Funakoshi Co., Ltd. make), pAGE107 [JP,3-22979,A;Cytotechnology, 3, and 1 33 (1990)], pAS 3-3 (JP,2-227075,A) and pCDM8 [Nature, 329, and 840 (1987)], pcDNAI/A mp (product made from Invitrogen), pREP4 (product made from Invitrogen) and pAGE103 [J.Biochemistry, 101, and 1307 (1987)], and pAGE210 grade can be mentioned.

[0083] As a promotor, if it can be discovered in an animal cell, all can be used, for example, the promotor of IE (immediate early) gene of a cytomegalovirus (CMV), the initial promotor of SV40, the promotor of a retrovirus, a metallothionein promotor, a heat shock promotor, SRalpha promotor, etc. can be mentioned. Moreover, the enhancer of Homo sapiens's CMV IE gene may be used with a promotor.

[0084] As a host cell, the NAMARUBA (Namalwa) cell which is a human cell, the COS cell which is a cell of an ape, the CHO cell which is a cell of a Chinese hamster, HBT5637 (JP,63-299,A), etc. can be mentioned. If it is the approach of introducing DNA into an animal cell as the introductory approach of a recombination vector, all can be used, for example, the electroporation method [Cytotechnology, 3, and 133 (1990)], a calcium phosphate method (JP,2-227075,A), the RIPOFE cushion method [Proc.Natl.Acad.Sci.USA, 84, and 7413 (1987)], etc. can be mentioned.

[0085] When using an insect cell as a host, the polypeptide of this invention can be discovered by the approach indicated by the current PUROTO call Inn molecular biology supplement 1-38 (1

987-1997), Baculovirus Expression Vectors, A Laboratory Manual, W.H.Freeman and Company, NewYork (1992), Bio/Technology, 6, 47, etc. (1988).
[0086] That is, after carrying out cointroduction of a recombination gene installation vector and the baculovirus to an insect cell, rearranging in insect cell culture supernatant liquid and obtaining a virus, it can rearrange further, a virus can be infected with an insect cell, and the polypeptide of this invention can be made to discover. As a transgenics vector used in this approach, pVL1392, pVL1393, pBlueBacIII (both product made from Invitorogen), etc. can be mentioned, for example.
[0087] As a baculovirus, the out GURAFA KARIFORUNIKA NUKUREA poly sludge cis- virus (Autographa californica nuclear polyhedrosis virus) which is a virus infected with the department insect of a cutworm can be used, for example. As an insect cell, Sf9 and Sf21 which are the ovarian cell of Spodoptera frugiperda [Baculovirus Expression Vectors, A Laboratory Manual, W.H.Freeman and Company, and New York] (1992), High5 (product made from Invitrogen) which is the ovarian cell of Trichoplusia ni can be used.
[0088] As the cointroduction approach of of the above-mentioned recombination gene installation vector to an insect cell and the above-mentioned baculovirus for preparing a recombination virus, a calcium phosphate method (JP,2-2270,A 75), the RIPOFE cushion method [Proc.Natl.Acad.Sci.USA, 84, 7413 (1987)], etc. can be mentioned, for example. When using a plant cell as a host cell, a Ti plasmid, a tobacco mosaic virus vector, etc. can be mentioned as an expression vector.
[0089] As a promotor, if it can be discovered in a plant cell, which thing may be used, for example, 35S promotor of a cauliflower mosaic virus (CaMV), rice actin 1 promotor, etc. can be mentioned. As a host cell, plant cells, such as tobacco, a potato, a tomato, a ginseng, soybeans, rape, alfalfa, a rice, wheat, and a barley, etc. can be mentioned.
[0090] If it is the approach of introducing DNA into a plant cell as the introductory approach of a recombination vector, all can be used, for example, Agrobacterium (Agrobacterium) (JP,59-140885,A, JP,60-70080,A, WO 94/00977), the electroporation method (JP,60-251887,A), the approach (the 2606856th patent 2517813rd of a patent) using party Kurgan (gene gun), etc. can be mentioned.
[0091] As the gene expression approach, secretory production, a fusion polypeptide manifestation, etc. can be performed according to the approach indicated by the 2nd edition of molecular cloning in addition to a direct manifestation. When it is made discovered by yeast, the animal cell, the insect cell, or the plant cell, the polypeptide to which sugar or a sugar chain was added can be obtained.
[0092] This polypeptide can be manufactured by cultivating the transformant incorporating DNA of this invention which rearranges and holds an expression vector to a culture medium, carrying out generation are recording of the polypeptide of this invention into a culture, and extracting this polypeptide from this culture. As a culture medium which cultivates the transformant obtained considering eukaryotes, such as procaryotes, such as Escherichia coli, or yeast, as a host, the carbon source in which this living thing can carry out utilization, a nitrogen source, mineral, etc. are contained, and as long as it is the culture medium which can cultivate a transformant efficiently, any of a natural medium and a synthetic medium may be used.
[0093] Alcohols, such as organic acids, such as carbohydrates, such as a glucose, fructose, a sucrose, molasses containing these, starch, or starch hydrolysate, an acetic acid, and a propionic acid, ethanol, and propanol, etc. can be used that what is necessary is just that in which this living thing can carry out utilization as a carbon source. As a nitrogen source, the ammonium salt of inorganic acids, such as ammonia, an ammonium chloride, an ammonium sulfate, ammonium acetate, and ammonium phosphate, or an organic acid, other nitrogen-containing compounds and a peptone, a meat extract, a yeast extract, corn steep liquor, casein hydrolysate, soybean cake and soybean cake hydrolyzate, various fermentation fungus bodies, the digest of those, etc. can be used.
[0094] As mineral salt, the first potassium of a phosphoric acid, the second potassium of a phosphoric acid, magnesium phosphate, magnesium sulfate, a sodium chloride, a ferrous sulfate, a manganese sulfate, a copper sulfate, a calcium carbonate, etc. can be used. Culture is usually

performed under aerobic conditions, such as shaking culture or deep part aeration spinner culture. Culture temperature has good 15-40 degrees C, and culture time amount is usually for 16 hours - seven days. pH under culture is held to 3.0-9.0. Adjustment of pH is performed using an inorganic or organic acid, an alkali solution, a urea, a calcium carbonate, ammonia, etc.
[0095] Moreover, antibiotics, such as ampicillin and a tetracycline, may be added to a culture medium if needed during culture. When cultivating as a promotor the microorganism using an inductive promotor which was rearranged and carried out the transformation by the vector, an inducer may be added to a culture medium if needed. For example, when cultivating the microorganism which used the trp promotor for isopropyl-beta-D-thio galactopyranoside (IPTG) etc. when cultivating the microorganism using a lac promotor which was rearranged and carried out the transformation by the vector and which was rearranged and carried out the transformation by the vector, the Indore acrylic acid (IAA) etc. may be added to a culture medium.
[0096] As a culture medium which cultivates the transformant obtained considering the animal cell as a host RPMI1640 culture medium currently generally used [The Journal of the American Medical Association, 199, and 519 (1967)], The MEM culture medium of Eagle [Science, 122, and 501 (1952)], A Dulbecco alteration MEM culture medium [Virology, 8, and 396 (1959)], The culture medium which added fetal calf serum etc. can be used for 199 culture media [Proceeding of the Society for the Biolog ical Medicine, 73, and 1 (1950)] or these culture media. culture -- usually -- pH 6-8, 30-40 degrees C, and 5%CO -- it carries out for one - seven days under lower conditions 2 ****. Moreover, antibiotics, such as a kanamycin and penicillin, may be added to a culture medium if needed during culture.
[0097] As a culture medium which cultivates the transformant obtained considering the insect cell as a host, the TNM-FH culture medium (product made from Pharmingen) currently generally used, a Sf-900 II SFM culture medium (product made from Life Technologies), ExCell400 and ExCell405 (all are the products made from JRH Biosciences), Grace's Insect Medium [Nature, 195, and 788 (1962)], etc. can be used. Culture is usually performed for one - five days under conditions, such as pH 6-7 and 25-30 etc. degrees C. Moreover, antibiotics, such as gentamycin, may be added to a culture medium if needed during culture.
[0098] A plant cell can be made to be able to specialize in the cell and organ of the vegetation as a cell, and the transformant obtained as a host can cultivate it. As a culture medium which cultivates this transformant, auxin, cytokinin, etc. can use the culture medium which added plant hormone for Murashige - currently generally used and - SUKUGU (MS) culture medium, the White (White) culture medium, or these culture media. Culture is usually performed for three - 60 days under pH 5-9 and 20-40-degree C conditions. Moreover, antibiotics, such as a kanamycin and hygromycin, may be added to a culture medium if needed during culture.
[0099] This approach can be chosen by there being an approach which it makes host intracellular produce, an approach of making it secrete out of a host cell, or the approach of making it produce on a host cell envelope as a process of the polypeptide of this invention, and changing the host cell to be used and the structure of a polypeptide made to produce. When the polypeptide of this invention is produced on host intracellular or a host cell envelope, Paulson's and others approach [J.Biol.Chem., 264, and 17619 (1989)], Approach [Proc.Natl.Acad.Sci.USA of a low and others, 86, and 8227 (1989), This polypeptide can be made to secrete positively out of a host cell by applying the approach of a publication correspondingly to Genes Develop., 4, 1288 (1990)] or JP,5-336963,A, and WO94 / 23021 grades.
[0100] That is, the polypeptide of this invention can be made to secrete positively out of a host cell by making it discovered in the form which added transit peptide before the polypeptide including the active site of the polypeptide of this invention using the transgenic technique. Moreover, according to the approach indicated by JP,2-227075,A, a volume can also be raised using the gene amplification system using a dihydrofolate reductase gene etc.
[0101] Furthermore, by making the cell of the animal which carried out transgenics, or vegetation redifferentiate, the animal individual (transgenic nonhuman animal) or vegetable individual (transgenic plant) into which the gene was introduced can be developed, and the polypeptide of this invention can also be manufactured using these individuals. When a transformant is an

animal individual or a vegetable individual, this polypeptide can be manufactured by breeding or growing, carrying out generation are recording of this polypeptide according to the usual approach, and extracting this polypeptide from this animal individual or a vegetable individual.
[0102] The method of producing the polypeptide of this invention is mentioned into the animal which introduced and developed the gene as an approach of manufacturing the polypeptide of this invention using an animal individual, for example according to the well-known approach [American Journal of Clinical Nutrition, 63, 639S (1996), American Journal of Clinical Nutrition, 63, 627S (1996), Bio/Technology, 9, and 830 (1991)].
[0103] In the case of an animal individual, this polypeptide can be manufactured by breeding the transgenic nonhuman animal which introduced DNA which carries out the code of the polypeptide of this invention, generating and storing up this polypeptide into this animal, and extracting this polypeptide from the inside of this animal. As an are recording location in this animal, the milk (JP,63-309192,A) of this animal, an egg, etc. can be mentioned, for example. under the present circumstances -- although all can be used as a promotor boiled and used if it can be discovered for an animal -- an alveolar epithelial cell -- specific alpha casein promotor who is a promotor, beta casein promotor, a beta lactoglobulin promotor, a whey acidity protein promotor, etc. are used suitably.
[0104] As an approach of manufacturing the polypeptide of this invention using a vegetable individual For example, well-known approach [tissue culture and 20 (1994), the transgenic plant which introduced DNA which carries out the code of the polypeptide of this invention It grows according to tissue culture, 21 (1995), Trends in Biotechnology, 15, and 45 (1997)]. The method of producing this polypeptide is mentioned by generating and storing up this polypeptide into this vegetation, and extracting this polypeptide from the inside of this vegetation.
[0105] When the polypeptide of this invention is discovered in the state of the dissolution to intracellular, the polypeptide manufactured by the transformant of this invention collects cells according to centrifugal separation after culture termination, crushes a cell by the ultrasonic crusher, the French press, the MANTONGAURIN homogenizer, dynomill, etc. after suspending in the drainage system buffer solution, and obtains a cell-free extract. The isolation purification method of an enzyme usual from the supernatant liquid obtained by carrying out centrifugal separation of this cell-free extract, Namely, the salting-out method by the solvent extraction method, an ammonium sulfate, etc., the desalting method, settling by the organic solvent, The anion-exchange chromatography method using resin, such as diethylaminoethyl (DEAE)-sepharose and DIAIONHPA-75 (Mitsubishi Kasei Corp. make), The cation-exchange chromatography method using resin, such as S-Sepharose FF (product made from Pharmacia), The hydrophobic chromatography method using resin, such as butyl sepharose and phenyl sepharose, independent in technique, such as electrophoresis methods, such as gel filtration using molecular sieving, the affinity chromatography method, the chromatofocusing method, and isoelectric focusing, -- or it can combine and use and a purification preparation can be obtained.

[0106] Moreover, when this polypeptide forms an insoluble object in intracellular and is discovered, the insoluble objects of a polypeptide are collected as a precipitate fraction by crushing after collecting cells similarly and performing centrifugal separation. The collected insoluble object of a polypeptide is solubilized with a protein modifier. After returning this polypeptide to a normal spacial configuration by diluting or dialyzing this solubilization liquid, the purification preparation of this polypeptide can be obtained according to the same isolation purification method as the above.
[0107] When derivatives, such as a polypeptide of this invention or its sugar qualification object, are secreted out of a cell, derivatives, such as this polypeptide or its sugar chain adduct, can be collected to a culture supernatant. That is, a purification preparation can be obtained by acquiring a soluble fraction and using the same isolation purification method as the above from this soluble fraction by processing this culture by technique, such as the same centrifugal separation as the above.
[0108] moreover, the polypeptide of this invention -- Fmoc -- law (fluorenyl methyloxy carbonyl process) and tBoc -- it can manufacture also by chemosynthesis methods, such as law (t-

butyloxy carbonyl process). Moreover, chemosynthesis can also be carried out using peptide synthesis machines, such as Advanced ChemT ech, Perkin-Elmer, Amersham Pharmacia Biotech, Protein Tec hnology Instrument, Synthecell-Vega, PerSeptive, and Shimadzu.
[0109] 4. Antibodies which recognize the polypeptide of this invention, such as a polyclonal antibody and a monoclonal antibody, are producible by using as an antigen the synthetic peptide which has some amino acid sequences of the purification preparation of the partial fragment polypeptide of the polypeptide of preparation this invention of the antibody which recognizes the polypeptide of this invention, or this polypeptide, or the polypeptide of this invention.
[0110] (1) A polyclonal antibody is producible by medicating the inside of hypodermically [of an animal], and a vein, or intraperitoneal with a suitable adjuvant (for example, [Freund's complete adjuvant (Complete Freund's Adjuvant) or aluminium hydroxide gel, a pertussis vaccine], etc.), using as an antigen the peptide which has some amino acid sequences of the overall length of the polypeptide of production this invention of a polyclonal antibody, the purification preparation of the partial fragment polypeptide of this polypeptide, or the polypeptide of this invention.
[0111] As an animal prescribed for the patient, a rabbit, a goat, the rat of three to 20 weeks old, a mouse, a hamster, etc. can be used. The dose of this antigen has desirable 50-100microper animal g. When using a peptide, it is desirable to use as an antigen what carried out covalent bond of the peptide to carriea protein, such as a SUKASHI guy hemocyanin (keyhole limpet haemocyanin) and cow thyroglobulin. The peptide used as an antigen is compoundable with a peptide synthesis machine.
[0112] Administration of this antigen is performed 3 to 10 times every one - two weeks after the 1st administration. It will collect blood from an eyegrounds venous plexus after each administration on three - the 7th, and will check that this blood serum reacts with the antigen used for immunity with enzyme immunoassay [enzyme immunoassay (ELISA method):********** (1976), Antibodies-A Laboratory Manual, and Cold Spring Harbor Laboratory (1988)] etc.
[0113] The blood serum can acquire a blood serum from the nonhuman mammal which showed sufficient antibody titer to the antigen used for immunity, and a polyclonal antibody can be acquired by separating and refining this blood serum. As an approach of separating and refining, independent or the approach of combining and processing is mentioned in the chromatography using centrifugal separation, the salting-out by 40 - 50% saturation ammonium sulfate, caprylic-acid precipitate [Antibodies, A Laboratory manual, and Cold SpringHarbor Laboratory (1988)] or a DEAE-sepharose column, an anion-exchange column, protein A, G-column, or a gel filtration column etc.
[0114] (2) Offer the rat which the blood serum showed sufficient antibody titer as a source of supply of an antibody forming cell to the partial fragment polypeptide of the polypeptide of this invention used for the preparation immunity of (Production a) antibody sexuparaous cell of a monoclonal antibody. A spleen will be extracted on three - the 7th, after carrying out the last administration of the antigen matter at the rat which showed this antibody titer.
[0115] Beating of this spleen is carried out in an MEM culture medium (NISSUI PHARMACEUTICAL CO., LTD. make), and it unfolds with pincettes, and supernatant liquid is thrown away after carrying out at-long-intervals alignment separation by 1,200rpm for 5 minutes. After processing the splenic cells of the obtained precipitate fraction for 1 - 2 minutes with the tris-ammonium-chloride buffer solution (pH7.65) and removing an erythrocyte, it washes 3 times by the MEM culture medium, and the obtained splenic cells are used as an antibody forming cell.
[0116] (b) Use the established cell line acquired from the mouse or the rat as a preparation myeloma cell of a myeloma cell. For example, 8-azaguanine resistance mouse (BALB/c origin) myeloma cell stock P3-X63Ag8-U1 [Curr.Topics.Microbiol.Immunol., 81, and 1 (1978), (It abbreviates to P3-U1 hereafter) Europ.J.Immunol., 6, 511 (1976)], SP2 / 0-Ag14 (SP-2) [Nature, 276, and 269 (1978)], P3-X63-Ag8653 (653) [J.Immunol., 123, and 1548] (1979) P3-X63-Ag8 (X63) [Nature, 256, and 495 (1975)] etc. can be used. These cell strains to 8-azaguanine culture-medium [RPMI-1640 culture medium A glutamine (1.5 mmol/l), Although a passage is carried out by culture-medium] which added 8-azaguanine (15microg/(ml)) to the culture medium (henceforth a normal culture medium) which added 2-mercaptoethanol (5x10-5 mol/l), JIENTA mycin (10microg/(ml)), and fetal calf serum (FCS) (CSL company make, 10%) further It cultivates

by the normal culture medium three - four days before cell fusion, and these 2x107 or more cells are used for fusion.
[0117] (c) Throw away supernatant liquid after an MEM culture medium or PBS (1.83g [of phosphoric-acid disodium] and phosphoric-acid 1 potassium 0.21g, 7.65g of salt, 1l. of distilled water, pH7.2) is sufficient, and washing the antibody forming cell acquired by production (b) of a hybridoma, and the myeloma cell acquired by (b), mixing so that the number of cells may be set to antibody forming cell:myeloma cell =5-10:1, and carrying out at-long-intervals alignment separation by 1,200rpm for 5 minutes.
[0118] Unfolding the cell population of the obtained precipitation fraction well, and stirring to this cell population, at 37 degrees C, 0.2-1ml of solutions which mixed per 108 antibody forming cells, polyethylene-glycol-1000(PEG-1000) 2g, MEM 2ml, and dimethyl sulfoxide (DMSO) 0.7ml is added, and 1-2ml of MEM culture media is added several times for for [every] further 1 - 2 minutes.
[0119] After addition, it prepares so that an MEM culture medium may be added and the whole quantity may be set to 50ml. Supernatant liquid is thrown away for this preparation liquid after 5-minute alignment separation at long intervals by 900rpm. After unfolding the cell of the obtained precipitate fraction gently, it depends and absorbs to a measuring pipet, and blows off and appears in it, and it is gently suspended in HAT-medium [culture medium which added hypoxanthine (10-4 mol/l), thymidine (1.5x10-5 mol/l), and aminopterin (4x10-7 mol/l) to normal culture medium] 100ml.
[0120] This suspension is poured distributively 100microl / hole every on the plate for 96 hole culture, and it cultivates for seven - 14 days at 37 degrees C among 5% CO2 incubator. The hybridoma which reacts to the partial fragment polypeptide of the polypeptide of this invention specifically is chosen after culture with the enzyme immunoassay which takes a part of culture supernatant and is stated to anti BODIIZU [Antibodies, A Laboratorymanual, Cold Spring Harbor Laboratory, and Chapter 14 (1988)] etc.
[0121] The following approaches can be mentioned as a concrete example of enzyme immunoassay. The coat of the partial fragment polypeptide of the polypeptide of this invention used for the antigen is carried out to a suitable plate in the case of immunity. The purification antibody obtained by the hybridoma culture supernatant or the below-mentioned (d) is made to react as the first antibody. After making the anti-rat or anti-mouse immunoglobulin antibody which furthermore carried out the indicator with a biotin, an enzyme, the chemiluminescence matter, or a radiation compound as the second antibody react, the reaction according to a marker is performed. What reacts to the polypeptide of this invention specifically is chosen as a hybridoma which produces the monoclonal antibody of this invention.
[0122] The thing repeats cloning twice by limiting dilution, and [uses 1st HT culture medium (culture medium excluding aminopterin from the HAT medium), and uses the 2nd normal culture medium] and in which it was stabilized and strong antibody titer was accepted is chosen as a hybridoma stock which produces the monoclonal antibody of this invention using this hybridoma.
(d) Inject intraperitoneal with the 20x106 cell / [the monoclonal antibody production hybridoma cell 5 -] ** to the polypeptide of this invention acquired by (c) to the mouse of eight to 10 weeks old or nude mouse which carried out preparation pristane processing [2, 6, 10, and 14-tetramethyl pentadecane (Pristane) 0.5ml are injected intraperitoneally, and it breeds for two weeks] of a monoclonal antibody. A hybridoma is ascites-tumor-ized in ten - 21 days.
[0123] Ascites is extracted from this ascites-tumor-ized mouse, at-long-intervals alignment separation is carried out by 3,000rpm for 5 minutes, and solid content is removed. A monoclonal antibody can be refined and acquired from the obtained supernatant liquid by the approach used by the polyclonal, and the same approach. The decision of the subclass of an antibody is made using a mouse monoclonal antibody typing kit or a rat monoclonal antibody typing kit. The amount of protein is computed from a Lowry method or the absorbance in 280nm.
[0124] 5. State the method of preparation of the recombination virus vector for producing the polypeptide of this invention in specific human tissue to below the method of preparation of the recombination virus vector which produces the polypeptide of this invention. The DNA fragment of the suitable die length which contains a code part [polypeptide / this] if needed based on

the perfect length cDNA of DNA of this invention is prepared.
[0125] A recombination virus vector is developed by inserting the perfect length cDNA or this DNA fragment in the lower stream of a river of the promotor in a virus vector. In the case of an RNA virus vector, a recombination virus is developed by adjusting a homologous RNA fragment to the DNA fragment of the suitable die length which contains in the perfect length cDNA of DNA of this invention the part which carries out the code of homologous cRNA or this polypeptide, and inserting them in the lower stream of a river of the promotor in a virus vector. An RNA fragment chooses one of the single strands of a sense chain or an antisense strand according to the class of virus vector besides 2 chains. For example, in the case of a Sendai Virus vector, homologous RNA is conversely chosen as an antisense strand for RNA which carries out homologous of the case of a retrovirus vector to a sense chain.
[0126] This recombination virus vector is introduced into the packaging cell which suited this vector. All the cells that can supply the polypeptide to which the recombination virus vector which is missing in at least one of the DNA which carries out the code of the polypeptide which needs a packaging cell for PAKKEJI-NGU of a virus this suffers a loss can be used, for example, can use HEK293 cell of the Homo sapiens kidney origin, mouse fibrocyte NIH3 T3, etc. As a polypeptide supplied in a packaging cell In the case of a retrovirus vector, gag of the mouse retrovirus origin, In the case of a lentivirus vector, polypeptides, such as pol and env, gag of the HIV origin, Polypeptides, such as pol, env, vpr, vpu, vif, tat, rev, and nef, In the case of an adenovirus vector, polypeptides, such as E1A of the adenovirus origin and E1B In the case of an adeno-associated virus, polypeptides, such as Rep (p5, p19, p40) and **** (Cap), are mentioned, and, in the case of Sendai Virus, polypeptides, such as NP, P/C, and L, M, F, HN, are mentioned.
[0127] As a virus vector, it rearranges in the above-mentioned packaging cell, a virus can be produced, and the thing containing a promotor is used for the location which can imprint DNA of this invention by the target cell. As a plasmid vector, MFG [Proc.Natl.Acad.Sci.USA, 92, and 6733-6737 (19 95)], pBabePuro [Nucleic Acids Res., 18, and 3587-3598 (1990)], LL-CG, CL-CG, CS-CG, and CLG [Journal of Virology, 72, and 8150-8157 (1998)], pAdex1 [Nucleic Acids Res., 23, and 3818-3821 (1995)] etc. is used.
[0128] As a promotor, if it can be discovered all over human tissue, all can be used, for example, the promotor of IE (immediateearly) gene of a cytomegalovirus (Homo sapiens CMV), the initial promotor of SV40, the promotor of a retrovirus, a metallothionein promotor, a heat shock protein promotor, SRalpha promotor, etc. can be mentioned. Moreover, the enhancer of Homo sapiens's CMV IE gene may be used with a promotor.
[0129] As a method of introducing the recombination virus vector to a packaging cell, a calcium phosphate method [JP,2-227075,A], the RIPOFE cushion method [Proc.Natl.Acad.Sci.U SA, 84, and 7413 (1987)], etc. can be mentioned, for example.
6. A structural change of the amount of mRNA manifestations of DNA of this invention in a specimen and this mRNA is detectable using DNA of approach this invention which detects the manifestation of DNA of use (1) this invention of DNA of this invention, a polypeptide, or an antibody.
[0130] The organization which acquired from the patient and healthy person who have as a specimen the disease from which manifestation change of DNA of this invention is the cause, Biological materials, such as a blood serum and saliva, the primary culture cell sample which acquired the cell from this biological material and was cultivated in the suitable culture medium in a test tube, Or mRNA or all RNA acquired from what isolated the organization which acquired from the biological material as paraffin or a cryostat intercept is used (this mRNA and all RNA are henceforth called the specimen origin RNA).
[0131] As an approach of detecting, approaches, such as a (1) Northern-blot-technique (2) in situ hybridization method, (3) quantitive PCR method, (4) differential hybridization method [Trends in Genetics 7 and 314 (1991)], (5) DNA-chip method [Genome Research, 6, and 639 (1996)], and the (6) RNase protection assay method, etc. are mentioned, for example. Hereafter, each detecting method is explained in full detail.
[0132] ** Imprint the Northern blot technique specimen origin RNA to base materials, such as a nylon filter, after separation by gel electrophoresis. Hybridization and washing are performed

after an imprint using the indicator probe prepared from DNA of this invention. The band of RNA specifically combined with this probe is detected after washing. By comparing this detection result with a healthy person about the specimen RNA of the patient origin, the amount of manifestations of this RNA and change of structure are detectable. In case hybridization is performed, mRNA made into the purpose under a probe and specimen origin RNA carries out an incubation on the conditions which form a stable hybrid. the approach of an edition [of molecular cloning / 2nd] publication of hybridization and a washing process in order to prevent false positivity -- applying correspondingly -- quantity -- it is desirable to carry out on stringent conditions.

[0133] The indicator probe used for a Northern blot technique can be prepared by making the oligonucleotide which designed the radioisotope, the biotin, the fluorescence radical, the chemiluminescence radical, etc. from the array of DNA of this invention, or this DNA by the well-known approach (nick translation, a random priming, or KINAJINGU), for example incorporate. The amount of association to mRNA of an indicator probe can carry out the quantum of the amount of manifestations of this mRNA by carrying out the quantum of the amount of the united indicator probe from reflecting the amount of manifestations of this mRNA. Moreover, a structural change of this mRNA can be known by analyzing the part on the filter which an indicator probe combines.

[0134] **in Perform hybridization and the process of washing using the specimen which isolated the organization which acquired from the situ hybridization method living body as paraffin or a cryostat intercept, and was obtained, and an indicator probe given in **. The amount of manifestations of mRNA specifically combined with this probe by the same approach as ** is detectable after washing. in the approach indicated by current PUROTO call Inn molecular biology etc. in hybridization and a washing process by the situ hybridization method in order to prevent false positivity -- applying correspondingly -- quantity -- it is desirable to carry out on stringent conditions.

[0135] ** Target RNA is detectable by using the approach based on compounding cDNA using the quantitive PCR method specimen origin RNA, an oligo dT primer or a random primer, and reverse transcriptase (this cDNA is henceforth called the specimen origin cDNA). When the specimen origin RNA is mRNA, any primer of the above-mentioned ** can be used, but when these specimen origins RNA are all RNA, it is required to use an oligo dT primer.

[0136] At the quantitive PCR method, the DNA fragment of the specific mRNA origin is amplified by performing PCR using the primer designed based on the base sequence which makes the specimen origin cDNA a template and DNA of this invention has. Since the amount of this magnification DNA fragment reflects the amount of manifestations of this mRNA, it can carry out the quantum of the amount of this mRNA by placing DNA which carries out the code of an actin, G3 PDH (glyceraldehyde 3-phosphate dehydrogenase), etc. as internal control. Moreover, change of the structure of this mRNA can also be known by separating this magnification DNA fragment by gel electrophoresis. It is desirable to use the suitable primer which amplifies a target sequence specifically and efficiently by this detecting method. Neither association between primers nor association in a primer can be caused, but it can combine with Target cDNA specifically at annealing temperature, and a suitable primer can be designed based on conditions, such as shifting, from Target cDNA on denaturation conditions. The quantum of a magnification DNA fragment needs to carry out to the inside of the PCR reaction which the magnification product is increasing exponentially. Such an PCR reaction can be known by collecting these magnification DNA fragments produced for every reaction, and carrying out quantitative analysis by gel electrophoresis.

[0137] ** Perform hybridization and washing to the base of the filter or slide glass which made DNA of this invention fix, silicon, etc. by using as a probe the specimen origin cDNA prepared by the approach indicated by differential hybridization method and DNA chip method **. Fluctuation of the amount of manifestations of mRNA of this cDNA origin is detectable after washing by measuring the amount of cDNA(s) specifically combined with DNA of this invention. The difference in the manifestation of this mRNA between a contrast specimen and a target specimen is correctly detectable because any approach of a differential hybridization method and

a DNA chip method fixes internal control of an actin, G3 PDH, etc. on a filter or a base. Moreover, indicator cDNA composition can be performed using an indicator dNTP different, respectively based on a contrast specimen and RNA of the target specimen origin, and the quantum of the amount of manifestations of this exact mRNA can be performed by making the filter of one sheet, or the base of one sheet hybridize two indicator cDNA probes to coincidence.

[0138] ** Combine promotor arrays, such as T7 promotor and SP6 promotor, with 3' edge of DNA of RNase protection assay method this invention, and compound the antisense RNA which carried out the indicator using rNTP which carried out the indicator by the imprint system of in vitro using RNA polymerase. After combining this indicator antisense RNA with the specimen origin RNA and making a RNA-RNA hybrid form, it digests by RNase, and a band is made to form by gel electrophoresis and the RNA fragment protected from digestion is detected. By carrying out the quantum of the obtained band, the quantum of the amount of manifestations of mRNA combined with the above-mentioned indicator antisense RNA can be carried out.

[0139] In addition, the DNA fragment obtained from DNA or them which have the base sequence expressed with either of the array numbers 6-10, for example as DNA used for the approach indicated to either ** - ** is mentioned. moreover, as a specimen with which detection by the approach concerned is presented The disease accompanied by activation of unusual immunocytes, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, psoriasis, gout, various encephalomyelitis, The disease accompanied by infection and inflammation of congestive heart failure, traumatic brain injury, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, articular rheumatism, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome), Diseases, such as adult respiratory distress syndrome (ARDS:adultrespiratory distress syndrome), are mentioned, and it can use for a diagnosis of the above-mentioned disease by detecting the manifestation of DNA of this invention by the detection approach concerned.

[0140] (2) Describe how to detect the existence of the variation of DNA of this invention in a test subject, below the approach of detecting the variation of DNA of this invention. The variation of this DNA in a test subject is detectable by comparing directly by DNA and the following approach of this invention. From a test subject, the samples of the primary culture cell origin established from a Homo sapiens biological material or these biological materials, such as an organization, a blood serum, and saliva, are collected, and DNA is extracted out of this biological material or this primary culture cell origin sample (this DNA is hereafter called the specimen origin DNA). Or cDNA is acquired from mRNA of this sample origin with a conventional method (this cDNA is hereafter called the specimen origin cDNA). These specimen origins DNA and cDNA are used as mold, and DNA is amplified by the PCR method etc. using the primer designed based on the base sequence which DNA of this invention has. The obtained magnification DNA is used as a sample DNA.

[0141] The approach of detecting the heteroduplex formed as an approach of detecting whether variation being in Magnification DNA, of hybridization with the DNA strand which has a wild type allele, and the DNA strand which has variation allele can be used. The heteroduplex detecting method according to ** polyacrylamide gel electrophoresis in the approach of detecting a heteroduplex [Trends Genet., 7, and 5 (1991)], ** A single strand conformation polymorphism analysis method [Genomics, 16, and 325-332 (1993)], ** Chemical cleavage method (CCM, chemical cleavage of mismatches) [Human Molecular Genetics (1996) of a mismatch, Tom

Strachan and Andre w P.Read (BIOS Scientific Publishers Li mited)]. ** The enzyme-intercept method of a mismatch [Nature Genetics, 9, and 103-104 (1996)]. ** Denaturation gel-electrophoresis [Mutat.Res., The approach of 288, a 103-112 (1993)]** protein compaction trial (the protein truncation test:PTT method) [Genomics, 20, and 1-4 (1994)], etc. is mentioned. Hereafter, the above-mentioned approach is explained.

[0142] ** Amplify as a DNA fragment smaller than 200bp by the primer which designed the heteroduplex detecting method specimen origin DNA by polyacrylamide gel electrophoresis, or the specimen origin cDNA to the template based on the base sequence given [this DNA] in either of the array numbers 6-10. 2 chain formation processing by each magnification DNA fragment is performed with a conventional method using DNA of this invention, and this magnification DNA fragment of the test subject origin. Polyacrylamide gel electrophoresis is performed after processing. When a heteroduplex is formed of the variation of this DNA, mobility is later than a gay double strand without variation, and they can be detected as a band different from a gay double strand. It is better for degree of separation to use gels (Hydro-link, MDE, etc.) of special make. If it is retrieval of a fragment smaller than 200bp(s), insertion, deletion, and almost all 1 base substitution are detectable. As for heteroduplex analysis, it is desirable to carry out by the gel of one sheet combined with the single strand conformation polymorphism analysis described below.

[0143] ** Carry out electrophoresis of this DNA amplified as a fragment smaller than 200bp in native polyacrylamide gel after denaturalizing by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template at either of the array numbers 6-10 based on the base sequence of a publication in single strand conformation polymorphism analysis-method single strand conformation polymorphism analysis (SSCP analysis; single strand conformation polymorphism analys is). This amplified DNA is detectable as a band by carrying out the indicator of the primer by radioisotope or the fluorochrome, in case DNA magnification is performed, making this indicator into an index, or carrying out the argentation of the magnification product of a non-indicator after electrophoresis. A fragment with variation is detectable from the difference in mobility by carrying out electrophoresis of the magnification DNA fragment of the DNA origin of this invention, and the thing of the test subject origin to coincidence.

[0144] ** In the chemical cleavage method (the CCM method) of the chemical cleavage method mismatch of a mismatch, one chain of DNA of the location which is carrying out the mismatch by making DNA of this invention hybridize the DNA fragment amplified by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template based on the base sequence given [this DNA] in either of the array numbers 6-10 with the indicator DNA which made the radioisotope or the fluorochrome take in, and processing it with an osmium tetroxide can be made to be able to cut, and variation can be detected. The CCM method is one of the detecting methods sensibility is the highest, and can be adapted also for the specimen of the die length of kilobase.

[0145] ** A mismatch can also be cut in [combining with the T4 phage RIZORU base, the enzyme which participates in restoration of a mismatch by intracellular / like Endonuclease VII /, and RNaseA] enzyme instead of the enzyme-intercept method above-mentioned osmium tetroxide of a mismatch.

** Carry out electrophoresis of the DNA fragment amplified by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template at either of the array numbers 6-10 based on the base sequence of a publication using the gel which has the concentration gradient and temperature gradient of a chemical modifier in denaturation gel-electrophoresis denaturation gel electrophoresis (denaturing gradient gel electrophoresis:DGGE law). The amplified DNA fragment will move in the inside of gel to the location which denaturalizes to a single strand, and after denaturation will not move it. Since the mobility within the gel of DNA amplified in the case where there is nothing with the case where variation is in this DNA differs, it is possible to detect existence of variation. It is good to attach a Pori (G:C) terminal for raising detection sensitivity at each primer.

[0146] ** Protein compaction trial (the protein truncation te st:PTT method)

The phase shift mutation which produces the deficit of a polypeptide by this trial, splice site

mutation, and nonsense mutation are specifically detectable. the special primer which connected T7 promotor array and the eukaryote translational initiation sequence with the five prime end of DNA which has the base sequence expressed with the PTT method to either of the array numbers 6-10 -- designing -- this primer -- using -- the specimen origin RNA -- reverse transcription PCR (RT-PCR) -- cDNA is created by law. A polypeptide will be produced if an in vitro imprint and a translation are performed using this cDNA. When this polypeptide is migrated to gel, the variation which produces a deficit does not exist if it is in the location where the migration location of this polypeptide is equivalent to a perfect length polypeptide, but a deficit is in this polypeptide, this polypeptide can migrate in a location shorter than a perfect length polypeptide, and extent of a deficit can be known from this location.

[0147] When variation is detected by the above-mentioned approach, it is possible to determine the base sequence of the specimen origin DNA which has variation with a conventional method, and the specimen origin cDNA using the primer designed based on the base sequence which DNA of this invention has. In the case of the test subject in whom the specimen origin DNA or the specimen origin cDNA has a specific disease, the variation leading to this disease can be specified by analyzing the determined base sequence. Henceforth, it can use for a diagnosis of a disease by detecting this variation.

[0148] In detection of variation other than the variation in the coding region of DNA detected by the above-mentioned approach, it can detect by inspecting the intron near this DNA and in this DNA, and a non-coding region like a regulatory sequence. The disease resulting from the variation in a non-coding region can be checked by detecting the unusual size in the disease patient at the time of comparing with a contrast specimen according to the approach indicated above, or mRNA of an unusual volume.

[0149] Thus, about this DNA existence of the variation in a non-coding region was suggested saying, it can clone by using for either of the array numbers 6-10 DNA which has the base sequence of a publication as a probe of hybridization. It can search for the variation in a non-coding region according to one of above-mentioned approaches.

[0150] The found-out variation can be identified as SNPs (single nucleotide poly mol FIZUMU) with a chain with a disease by performing statistics processing according to the approach indicated by Handbook of Human Genetics Linkage.The John Hop kins University Press and Baltimore (1994). As a diagnosable test subject, by the approach of detecting the above-mentioned variation The disease accompanied by activation of unusual immunocytes, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, psoriasis, gout, various encephalomyelitis, The disease accompanied by infection and inflammation of congestive heart failure, traumatic brain injury, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, articular rheumatism, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemicinflammatory response syndrome), Those who have ones, such as adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome), of diseases can be mentioned.

[0151] (3) The approach antisense RNA / DNA technical [bioscience and the industry which control the imprint or translation of DNA which carries out the code of the polypeptide of this invention using DNA or the oligonucleotide of this invention, and 50,322 (1992), Chemistry, 46, 681 (1991), Biotechnology, 9, and 358 (1992), Trends in Biotechnology, 10, and 87 (1992), Trends in Biotechnology, 10, and 152 (1992), With a cell technology, 16, 1463 (1997)], a triple helix technique [Trends in Biotechnology, 10, and 132 (1992)], etc. The imprint or translation of DNA

which carries out the code of the polypeptide of this invention can be controlled using DNA of this invention. For example, the system (a living body is included) which can discover the polypeptide of this invention for DNA or the oligonucleotide of this invention is made to live together, and the manifestation of this polypeptide can be controlled on an imprint and translation level.

[0152] This control approach Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, traumatic brain injury, The disease accompanied by infection and inflammation of inflammatory bowel disease etc., a Burkitt lymphoma, Hodgkin's disease, The disease accompanied by unusual cell proliferations, such as various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as articular rheumatism and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic in flammatory response syndrome), The variation of DNA which carries out the code of the polypeptide of this invention can use adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc. for the therapy or prevention of a disease used as a cause.

[0153] (4) It is possible to acquire the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of this invention by the well-known approach [the volume the 2nd edition of molecular cloning and for University of Tokyo Institute of Medical Science carcinostatic research sections, a new cell technology experiment protocol, and Shujunsha (1993)], using as a probe DNA or the oligonucleotide of approach this invention which acquires the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of this invention using DNA or the oligonucleotide of this invention. For example, the thing of a rat or the Homo sapiens origin is acquirable by the following approaches.

[0154] It screens by approaches, such as plaque hybridization, to the genomic DNA library produced using the chromosome DNA isolated from cell and organization of a rat or Homo sapiens by using DNA or the oligonucleotide (especially 5' of cDNA near part) of this invention as a probe. The genomic DNA to hybridize is acquired by this screening. Promoterregion and imprint regulatory region can be obtained from this DNA. Moreover, an exon / intron structure can be clarified by comparing the base sequence of genomic DNA and the base sequence of cDNA which were acquired.

[0155] In addition, also in other nonhuman mammals, the promoterregion and the imprint regulatory region of this DNA are acquirable using the same approach. The field which participates in the basic imprint of DNA which carries out the code of the polypeptide of this invention in a mammalian cell as promoterregion is mentioned, and a field including an enhancer sequence, a silencer array which decreases which reinforces the basic imprint of DNA which carries out the code of the polypeptide of this invention as imprint regulatory region is mentioned. For example, the promoterregion and the imprint regulatory region which participate in the imprint of DNA which carries out the code of the polypeptide of this invention by human bone marrow can be mentioned. The promotor and imprint regulatory region which were obtained are applicable to the below-mentioned screening approach, and also they are useful in order to analyze the controlling mechanism of an imprint of this DNA.

[0156] (5) Various test compounds can be added to the cell strain of the approach patient origin which acquires the physic which controls the imprint of this DNA by screening using DNA which carries out the code of the polypeptide of this invention, and the matter which controls or promotes an imprint or translation of this DNA can be screened by authorizing the change in the manifestation of mRNA using DNA of this invention. The change in the manifestation of mRNA of

this DNA is detectable by the above-mentioned PCR method and the above-mentioned Northern blot technique, and the RNase protection assay method.

[0157] Various test compounds can be added to a patient origin cell strain, and the matter which promotes an imprint or translation of this DNA can be screened by authorizing the change in the manifestation of this polypeptide using the antibody which recognizes the polypeptide of this invention specifically. The change in the manifestation of this polypeptide is detectable by immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as the above-mentioned fluorescent antibody technique, enzyme immunoassay (the ELISA method), radioactive substance indicator immunity antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, the western blotting method, the dot blotting method, the immunoprecipitation method, and the sandwiches ELISA method.

[0158] The polypeptide of this invention on moreover, the lower stream of a river of the promoter region of DNA which carries out a code, and imprint regulatory region The reporter plasmid which connected the chloramphenicol acetyltransferase (CAT) gene and the luciferase gene as a reporter gene is built. After introducing into a suitable cell host and obtaining a transformant, the physic which controls by imprint level the manifestation of DNA which carries out the code of the polypeptide of this invention can be screened by adding various examined substances to the transformant, and analyzing the change in the manifestation of a reporter gene.

[0159] (6) How to acquire the physic which acts on the polypeptide of this invention by the screening approach using the polypeptide of this invention.

The physic which acts on the polypeptide of this invention can be screened by making the transformant which discovered the polypeptide of this invention, or the partial peptide of this polypeptide, and various examined substances live together, and analyzing fluctuation of activation of NF-kappa B in this transformant. Moreover, it can use for the medicinal screening to which the partial peptide of this refined polypeptide or this polypeptide also acts on this polypeptide specifically. The matter obtained by this screening is useful as physic for the therapy of the disease in which DNA and the polypeptide of this invention participated.

[0160] Hereafter, two sorts of screening procedures are explained.

Screening procedure (1)

The microorganism which carried out the transformation so that the polypeptide of this invention or the partial peptide of this polypeptide might be produced, an animal cell or an insect cell (the transformant for retrieval is called henceforth), and an examined substance are made to live together in an aquosity medium. According to the approach of a publication, the activity of NF-kappa B is measured after coexistence to above-mentioned 2. Microorganism, animal cell, or insect cell of the host who has not done a transformation is compared as a control group, and the target matter can be acquired by choosing the examined substance which fluctuates extent of activation of NF-kappa B in this transformant. Moreover, it can make into an index to check association to this transformant for retrieval of the compound specifically combined with this transformant for retrieval, or a polypeptide, and contention screening of the target compound can be carried out by the same approach as the above.

[0161] The polypeptide which constitutes a part of polypeptide of refined this invention or this polypeptide can be used for choosing the target compound specifically combined with this polypeptide. In order to carry out the quantum of the target compound, the polypeptide of this invention can be performed by the above-mentioned immunologic procedure using the antibody recognized specifically. Moreover, contention screening of the target compound can be carried out for checking association of the target compound combined with the polypeptide of this polypeptide or this polypeptide at an index.

[0162] Screening procedure (2)

Many peptides which constitute this a part of polypeptide can be compounded to high density on a plastics pin or a solid-state base material of a certain kind, and the compound or polypeptide alternatively combined with this peptide can be screened efficiently (WO 84/03564). In addition, the gene which receives transcriptional control by the polypeptide of this invention can be screened by analyzing gene expression using the transformant which discovers the polypeptide

of this invention.
[0163] (7) The gene therapy agent using the virus vector containing RNA which consists of DNA of this invention, DNA of gene therapy agent this invention containing RNA which consists of this DNA and a homologous array or this DNA, and a homologous array can be manufactured by preparing the basis which was produced by above-mentioned 5. and which is rearranged and is used for a virus vector and a gene therapy agent [Nat ure Genet., 8, and 42 (1994)]. If it is the basis usually used for injections as a basis used for a gene therapy agent, what kind of thing may be used and the mixed solution of amino acid solutions, such as sugar solutions, such as salting in liquid, such as mixture of distilled water, a sodium chloride or a sodium chloride, and mineral salt, a mannitol, a lactose, a dextran, and a glucose, a glycine, and an arginine, an organic-acid solution or salting in liquid, and a glucose solution etc. will be raised. Moreover, according to a conventional method, assistants, such as surfactants, such as vegetable oil, such as an osmotic-pressure regulator, pH regulator, sesame oil, and soybean oil, lecithin, or a nonionic surface active agent, may be used for these bases, and injections may be prepared as a solution, suspension, and dispersion liquid. these injections -- actuation of disintegration, freeze drying, etc. -- business -- the time -- as the pharmaceutical preparation for the dissolution -- it can also prepare . In the case of a liquid, the gene therapy agent of this invention remains as it is, and in the case of an individual, it can dissolve in the above-mentioned basis which carried out sterilization processing as occasion demands just before gene therapy, and can be used for a therapy. As a medication method of the gene therapy agent of this invention, the approach of prescribing for the patient locally can be raised so that it may be absorbed by a patient's therapy part.
[0164] A virus vector can be prepared by combining with an adenovirus vector the complex which produced complex combining the specific poly lysine-conjugate antibody in adenovirus hexone protein, and was obtained in DNA of suitable this invention of size. Stability is reached at a target cell, and it is incorporated by intracellular by endosome, and is decomposed by intracellular, and this virus vector can make DNA discover efficiently.
[0165] (-) The virus vector which used as the base Sendai Virus which is a chain RNA virus is also developed (Japanese Patent Application No. 9-517213, Japanese Patent Application No. 9-517214), and the Sendai Virus vector which incorporated KRGF-1 gene for the purpose of gene therapy can be produced. This DNA can be conveyed to the focus also by the non-virogene importing method.
[0166] By the well-known non-virogene importing method, in the field concerned A calcium phosphate coprecipitation method [Virology, 52, 456-467;(1973) Science, 209, and 1414-1422 (1980)], Microinjection method [Proc. Natl.Acad.Sci.USA, 77 and 5399-5403 1980 ;P roc.Natl.Acad.Sci.USA, 77, 7380-7384;(1980) Cell, 27, 223-231;(1981) Nature, 294, and 92-94 (1981) --] -- Liposome Minded membrane fusion-mediation importing method [Proc.Natl. Acad.Sci.USA and 84, 7413-7417;(1987) Biochemistry, 28, 9508-9514;(1989) J.Biol.Chem., 264, and 12128-12129;(1989) Hum.Gene T her. and 3,267-275 () 1992;Science and 249, Method [of 1285-1288;(1990) Circulation, 83 2007-2011 (1992)] or direct DNA incorporating, and acceptor-medium DNA importing] [Science, 247, and 1465-1468 ;J.Biol.Chem., (1990) 266 14338-14342 (1991) ;P roc.Natl.Acad.Sci.USA, 87, 3655-3659;(1991) J.Biol.Chem., 26 4 and 16985-16987 ; BioTechniques, (1989) 11 474-485 (1991) ;P roc. Natl.Acad.Sci.USA, 87 3410-3414 (1990) ;P roc. Natl.Acad.Sci.USA, 88 4255-4259 (1991) ;P roc. Natl.Acad.Sci.USA, 87 4033-4037 (1990) ;P roc.Natl.Acad.Sci.USA, 88, 8850-8854;(1991) Hum. Gene Ther., 3, 147-154(1991)], etc. can be mentioned.
[0167] By the membrane fusion-mediation importing method through liposome, it is reported in the research on a neoplasm by medicating with a liposome preparation object directly the organization which considers as a target that incorporation and manifestation of the organization concerned of a local gene are possible [Hum.Gene Ther., 3, and 399-410 (1992)]. Therefore, the same effectiveness is expected also by the disease focus in which DNA and the polypeptide of this invention participate. In order to carry out direct targetting of the DNA to the focus, a direct DNA incorporation technique is desirable. Acceptor-medium DNA import is performed for example, through the poly lysine by carrying out conjugate of the DNA (the gestalt of the

supercoiling plasmid which usually carried out the ring closure in share being taken) to polypeptide ligand. Ligand is chosen based on existence of the ligand acceptor to which it corresponds on a target cell or the cell surface of an organization. By request, a blood vessel can be directly injected with the ligand-DNA conjugate concerned, and it can point to it in the target tissue to which internalization of acceptor association and DNA-protein complex takes place. In order to prevent intracellular destruction of DNA, concurrent infection of the adenovirus can be carried out and an endosome function can also be collapsed.
[0168] (8) The organization containing the polypeptide or this polypeptide of this invention is immunologically detectable by making an antigen-antibody reaction perform using the antibody which recognizes specifically the polypeptide of approach this invention which detects the polypeptide of this invention immunologically using the antibody of this invention. This detecting method Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, traumatic brain injury, hypertrophic arthritis, psoriasis, gout, various encephalomyelitis, The disease, Burkitt lymphoma accompanied by infection and inflammation of congestive heart failure, inflammatory bowel disease, etc., The disease accompanied by unusual cell proliferations, such as Hodgkin's disease, various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as rheumatoid arthritis and fibroid lung, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome), The variation of DNA which carries out the code of the polypeptide of this invention can use adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc. for a diagnosis of the disease used as a cause. Moreover, this detection approach is used also for the quantum of a polypeptide.
[0169] as detection and an approach of carrying out a quantum, immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as a fluorescent antibody technique, enzyme immunoassay (the ELISA method), radioactive substance indicator immunity antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, a western blotting method, the dot blotting method, an immunoprecipitation method, the sandwiches ELISA method [a monoclonal antibody experiment manual (Kodansha -- scientific) (1987), New Biochemistry Experiment Lectures 5, and an immunobiochemistry approach (Tokyo Kagaku Dojin) (1986)], etc. are mentioned immunologically.
[0170] After a fluorescent antibody technique makes the antibody of this invention react to the microorganism, the animal cell, insect cell, or organization which discovered the polypeptide of this invention out of intracellular or a cell and makes the anti-mouse IgG antibody which carried out the label with fluorescent materials, such as fluorescin isothiocyanate (FITC), further, or its fragment react, it is the approach of measuring a fluorochrome with flow cytometer.
[0171] Enzyme immunoassay (the ELISA method) is the approach of measuring coloring coloring matter with an absorptiometer, after making the anti-mouse IgG antibody which the antibody of this invention was made to react to the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out of intracellular or a cell, and gave it enzyme labeling, such as a peroxidase and a biotin, etc. further, or a joint fragment react.
[0172] Radioactive substance indicator immunity antibody technique (RIA) is the approach of measuring with a scintillation counter etc., after making the anti-mouse IgG antibody which the antibody of this invention was made to react to the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out of intracellular or a cell, and gave it the radiation indicator further, or its fragment react. After an immunocyte staining technique and an immunity staining method make the antibody which recognizes this polypeptide specifically in the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out

of intracellular or a cell react and make the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescent materials, such as FITC, a peroxidase, and a biotin, further, or its fragment react, they are the approach of observing using a microscope.
[0173] The microorganism which discovered this polypeptide out of intracellular or a cell with the western blotting method, After carrying out fractionation of an animal cell, an insect cell, or the extract of an organization by SDS-polyacrylamide gel electrophoresis [Antibodies-A Laboratory Manual and Cold SpringHarbor Laboratory (1988)], Blotting of this gel is carried out to the PVDF film or a nitrocellulose membrane. After making the antibody which recognizes this polypeptide of this invention specifically react to this film and making the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescent materials, such as FITC, a peroxidase, and a biotin, further, or its fragment react, it is the approach of checking.
[0174] After the dot blotting method carries out blotting of the microorganism which discovered this polypeptide out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization to a nitrocellulose membrane, makes the antibody of this invention react to this film and makes the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescent materials, such as FITC, a peroxidase, and a biotin, further, or a joint fragment react, it is the approach of checking.
[0175] An immunoprecipitation method is an approach of adding the support which has a specific binding affinity to immunoglobulins, such as protein G-sepharose, and making an antigen antibody complex sedimenting, after making the microorganism which discovered the polypeptide of this invention out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization react with the antibody which recognizes this polypeptide specifically.
[0176] The sandwiches ELISA method is the antibody which recognizes the polypeptide of this invention specifically. The antibody which is one side beforehand among two kinds of antibodies from which an antigen recognition site differs is made to stick to a plate. The indicator of another antibody is carried out with enzymes, such as fluorescent materials, such as FITC, a peroxidase, and a biotin. After making the microorganism which discovered this polypeptide out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization react to an antibody adsorption plate, it is the approach of making the antibody which carried out the indicator reacting and performing the reaction according to a marker.
[0177] (9) It is useful to identify a structural change of the polypeptide which has changed and discovered the amount of manifestations of this polypeptide in the approach Homo sapiens biological material row Homo sapiens primary culture cell which diagnoses a disease using the antibody which recognizes the polypeptide of this invention specifically, when getting to know the danger of showing the symptoms of a disease in the future, and the cause of a disease whose symptoms were already shown. As an approach of detecting and diagnosing the amount of manifestations of this polypeptide, and a structural change, immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as the above-mentioned fluorescent antibody technique and the above-mentioned enzyme immunoassay (the ELISA method), radioactive substance indicator immunity antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, a western blotting method, the dot blotting method, an immunoprecipitation method, the sandwiches ELISA method, etc. are mentioned.
[0178] As a specimen with which the diagnosis by the above-mentioned approach is presented, allergy, atopy, The disease accompanied by activation of unusual immunocytes, such as asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, traumatic brain injury, hypertrophic arthritis, The disease accompanied by infection and inflammation of psoriasis, gout, various encephalomyelitis, congestive heart failure, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, rheumatoid arthritis, and fibroid lung, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on

the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome), Adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc., The cell and cell extract which were acquired from the biological material itself or these biological materials, such as the organization and blood which were acquired from the patient of the disease from which the variation of DNA which carries out the code of the polypeptide of this invention is the cause, a blood serum, urine, facilities, and saliva, are used. Moreover, what isolated the organization which acquired from the biological material as paraffin or a cryostat intercept can also be used.
[0179] The ELISA method and a fluorescent antibody technique using a microtiter plate as an approach of detecting immunologically, a Western blot technique, an immunity staining method, etc. are mentioned. The radioimmunoassay method using the antibody which recognizes the polypeptide of this invention and the polypeptide of this invention which carried out the indicator with radioisotopes using two kinds of monoclonal antibodies from which an epitope differs in the liquid phase as an approach of carrying out a quantum immunologically among the polypeptide of this invention and the antibody which reacts, such as the sandwiches ELISA method and 125I, etc. is mentioned.
[0180] (10) Use the recombination vector which comes to contain DNA of production this invention of a knock out nonhuman animal using DNA of this invention. In embryonic stem cells (embryonic stem cell), such as the target nonhuman animal, for example, a cow, a sheep, a goat, Buta, a horse, a mouse, and a fowl DNA which carries out the code of the polypeptide of this invention on a chromosome -- the technique of well-known homologous recombination -- [-- for example The variation clone permuted by the array of inactivation or arbitration by] (1987), such as Nature, 326, 295 (1987), Cell, 51, and 503, is produced ([Nature, 350, and 243] (1991)). [for example,] The chimera individual which consists of an embryonic stem cell clone and a normal cell can be prepared using the variation clone of an embryonic stem cell by technique, such as the impregnation chimera method to the blastocyst (blastcyst) of the fertilized egg of an animal, or the set chimera method. The individual which has the variation of arbitration by crossing of this chimera individual and a normal individual in DNA which carries out the code of the polypeptide of this invention on the chromosome of the cell of the whole body can be obtained, and the manifestation of DNA which carries out the code of the polypeptide of this invention can obtain a knock out nonhuman animal as a part or an individual controlled completely out of the gay individual by which variation went into the both sides of homologue by crossing of that individual further.
[0181] Moreover, it is also possible to produce a knock out nonhuman animal by introducing variation to the location of the arbitration of DNA which carries out the code of the polypeptide of this invention on a chromosome. For example, it is possible to also make the activity of the product change by a permutation, deletion, insertion, etc. carrying out a base all over the translation field of DNA which carries out the code of the polypeptide of this invention on a chromosome, and introducing variation. Moreover, it is possible by introducing the same variation to the manifestation regulatory region to also make extent of a manifestation, a stage, tissue specificity, etc. change. It is also still more possible to control a manifestation stage, a manifestation part, the amount of manifestations, etc. by combination with a Cre-loxP system more positively. the example [Cell, 87, and 131 7 (1996)] to which deletion of the purpose gene was carried out only in the field using the promoter discovered in a specific field with a brain as such an example, and the adenovirus which discovers Cre -- using -- the target stage -- an organ -- the example [Science, 278, and 5335 (1997)] to which deletion of the purpose gene was carried out specifically is known.
[0182] Therefore, the knock out nonhuman animal which can control a manifestation by the stage and organization of arbitration, or has insertion of arbitration, deletion, and a permutation in the translation field and manifestation regulatory region in this way also about DNA which carries out the code of the polypeptide of this invention on a chromosome is producible. A knock out nonhuman animal can guide the symptom of the various diseases resulting from the

polypeptide of this invention by the stage of arbitration, extent of arbitration, or the part of arbitration. Thus, the knock out nonhuman animal of this invention serves as very useful animal model in the therapy and prevention of various diseases resulting from the polypeptide of this invention. It is very useful especially as models for evaluation, such as the remedy, a prophylactic and functional food, and health food.

[0183] 7. As an approach of introducing variation into the variation installation this polypeptide of the polypeptide of variation installation of the polypeptide of this invention, and selection (1) this invention of a functional alteration variant, what kind of approach of deletion, insertion, and a permutation may be used. The deletion and insertion of a polypeptide are possible by carrying out deletion of this DNA fragment by the approach indicated by the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, etc. in DNA which carries out the code of this polypeptide, or making a suitable DNA fragment insert.

[0184] For example, it can obtain by graduating by DNA polymerase, such as Klenow Fragment (product made from TaKaRa), and making it re-connect after digestion, with this restriction enzyme of marketing of the plasmid which included a the same and different restriction enzyme site suitable in this DNA for a two-piece header and this DNA when it was a deletion mutant, if it is a flush end, if it is a cohesive end as it is. If it is an insertion variant, it can obtain by making double stranded DNA suitable after flush-end-izing insert and connect. A permutation variant is Error Prone as an approach of introducing variation at random. The PCR method [Trends In Biotechnology, 16, and 76 (1998)] etc. can be used. As an approach of introducing variation into the target location, the PCR method [Mutagenesis and Synthes is of Novel Recombinant Genes Using PCR, PCR PRIMER A LABORATORY MANUAL, 603 (1994)] or QuikChangeTMSite-Directed Mutagenesis Kit (product made from STRATAGENE) using a primer with variation etc. can be used.

[0185] (2) Selection of an activity rise alteration variant [as opposed to NF-kappa B activation according to the approach indicated to above-mentioned 2.] is more possible than the variant of this polypeptide produced by selection (1) of the functional alteration variant of the polypeptide of this invention. The functional alteration variant which went up the NF-kappa B activation function can be obtained by introducing each of the variant of this polypeptide and this polypeptide into a reporter cell, and specifically choosing the variant which raised reporter activity from this polypeptide. Moreover, a dominant negative variant can be obtained by choosing the variant of this polypeptide that controls NF-kappa B activation under the stimulus existence which activates NF-kappa B.

[0186] The variant of this polypeptide is introduced into a reporter cell, and, specifically, it is cytokine (TNF-alpha). T cell mitogen, such as TNF-beta, IL-1alpha, IL-1beta, IL-2, and LIF (an antigen stimulus) Lectin, an anti-T cell receptor antibody, anti-CD2 antibody, anti-CD3 antibody, anti-CD28 antibody, calcium ionophore, and B cell mitogen (an anti-IgM antibody --) anti-CD40, leukotriene, LPS and PMA, a parasitism somesthesis stain, virus infection (it CMV(s) HIV-1, HTLV-1, and HBV and EBV --) HSV-1, HHV-6, NDV, Sendai Virus, adenovirus, etc., A virus product (double stranded RNA, Tax and HBX, EBNA-2, LMP-1 grade), DNA destructive matter and protein synthesis inhibitor (for example, cycloheximide) A dominant negative variant can be obtained by giving the stimulus which activates NF-kappa B, such as ultraviolet rays, a radiation, and oxidation stress, and choosing the variant of this polypeptide which fell rather than the time of reporter activity having not introduced the variant.

[0187] In addition, the obtained dominant negative variant (Dominant Negative mutants; dominant functional control variant) can be applied to inflammation response control or growth control of a malignant cell, and may be able to use for the gene therapy of the disease accompanied by activation of NF-kappa B DNA which carries out the code of this dominant negative variant. An example is raised to below and this invention is explained concretely. However, these examples are the things for explanation and do not restrict the technical range of this invention.

[0188]

[Example] From the [example 1] Homo sapiens large intestine, the large intestine of the production Homo sapiens of a Homo sapiens fat tissue origin perfect length cDNA library, and fat tissue, mRNA was extracted [edition / 2nd / of molecular cloning] by the approach of a

publication. Furthermore, polyA+RNA was refined by oligo dT cellulose. The cDNA library was produced from each polyA+RNA with Oligo-capping method [Gene, 138, and 171-174 (1994)]. According to the approach of a publication, composition of BAP (Bacterial A lkaline Phosphatase) processing, TAP (Tobacco Acid Phosphatase) processing, RNA ligation, and the first chain cDNA and removal of RNA were performed to a protein nucleic-acid enzyme, 41, 197-201 or (1996) Gene, 200, and 149-156 (1997) using Oligo-cap linker (array number 11) and Oligo dT primer (array number 12). The double strand cDNA was amplified by having used the first obtained chain cDNA as mold by PCR using two sorts of primers, the sense primer by the side of a five prime end (array number 13), and the antisense primer by the side of a three-dash terminal (array number 14), and it cut by SfiI. The commercial kit:GeneAmp XL PCR kit (product made from Perkin Elmer) was used, for 1 minute was repeated at 95 degrees C after heat treatment for 5 minutes, it repeated [95 degrees C] the reaction cycle for 10 minutes 12 times for 1 minute and at 72 degrees C by 58 degrees C, and PCR performed it by holding at 4 degrees C after that.

[0189] The above-mentioned magnification cDNA was inserted in vector pME18SFL3 (GeneBank AB [009864], an expression vector, 3392bp) cut by DraIII, and the cDNA library was produced. About the plasmid DNA of each of the obtained clone, the base sequence of 5 'edge and 3' edge of cDNA DNA sequencing reagent () [Dye Terminator] Cycle SequencingFS Re ady Reaction Kit and dRhodamine Terminator Cycle Sequencing FS ReadyR eaction Kit or BigDye Terminator Cycle Sequencing FS Ready ReactionKit, and the product made from PE Biosystems are used. After performing a sequence reaction according to a manual, the base sequence was determined using the DNA sequencer (ABI PRISM 377, product made from PE Biosystems).

[0190] The artificial promotor who repeated the NF-kappa B recognition sequence in establishment IFN-beta of the reporter cell strain by which manifestation control of the luciferase activity is carried out by the [example 2] NF-kappa B enhancer (array number 15) 3 times was produced, and it inserted in 5' upstream region of the luciferase gene of a luciferase reporter vector (pAGE-luc; JP,3-22979,A, the experimental medicine, 7, and 96-103 (1989)) (it is henceforth called pIF-luc). This plasmid 4microg was dissolved in TE buffer solution [10 mmol/l tris-HCl (pH8.0), 1 mmol/l EDTA (ethylenediaminetetraacetic acid sodium)] so that it might be set to 1micro g/mu l, and transgenics was carried out to the Homo sapiens nephrocyte stock 293 (product made from Clontech) 1.6x106 piece by the electroporation method (the product made from BIO-RAD: Gene PulserTM). pIF-luc contains the hygromycin (Hygromycin) resistance gene, and after transgenics established the stabilization transformant for culture and hygromycin as a selective marker of transgenics by the RPMI culture medium [RPMI1640 (Nippon Suisan Kaisha, Ltd. make), 10% calf blood serum, 0.05 mmol/l-mercaptoethanol, 25 U/ml penicillin G, and 25U/ml streptomycin] which added hygromycin 0.2 g/l. Among stabilization transformant, by TNF-alpha stimulus, the stock which guided the high luciferase activity of 670 times as compared with no stimulating was chosen (it is henceforth called 293-/IF-LUC), and it used for the following manifestation assays.

[0191] Shaking culture of the clone which determined the base sequence in the analysis example 1 over NF-kappa B activation of the perfect length DNA using [example 3] 293 / IF-LUC was respectively carried out at 37 degrees C for 16 hours among 2ml (Yeast ex tract 10 g/l, Trypton 16 g/l, NaC 5 g/l) of 2xYT culture media which added ampicillin (100 mg/l). The centrifugal separator recovered the fungus body after culture, and the plasmid was respectively prepared by the approach of attachment data using the plasmid preparation kit (QIAPrep96 Turbo Miniprep Kit, product made from QIAGEN). It poured distributively so that it might become a plate with 20,000 per one well about 293 / IF-LUC cell 96 well, and it cultivated in the CO2 incubator at 37 degrees C for 16 hours. The RIPOFE cushion reagent (LIPOFECT AMINE 2000TM Reagent, product made from GIBCO BRL) was used for this cultured cell, respectively, and the 0.25micro of the above-mentioned plasmid abbreviation g was introduced into it according to the approach of attachment data. It used at 37 degrees C for 16 hours, a luciferase activity measurement reagent (LucLiteTM, product made from Packar) and luciferase activity measurement equipment (ARVO 1420 MULTILABEL COUNTER, product made from WALLC) were used after culture in the CO2 incubator, and luciferase activity was measured.

[0192] Consequently, COL03279 (DNA clone which has the base sequence of the array number 6), COL06772 (DNA clone which has the base sequence of the array number 7), ADKA01604 (DNA clone which has the base sequence of the array number 8), [when the plasmid of each clone of ADSU00701 (DNA clone which has the base sequence of the array number 9), and CAS01989 (DNA clone which has the base sequence of the array number 10) is introduced] As compared with negative control (pME18SFL3 is used), one 12.5 times, 6.3 times, 4.4 times, 2.7 times, and 3.0 times the activity of this was checked, respectively. DNA of this invention was respectively acquired from this clone.
[0193] the quantum of the amount of manifestations in the various organs of DNA of this invention accepted in each clone of the detection COL03279, COL06772, ADKA01604, and ADSU00701 of the amount of manifestations in the various organs of DNA of [example 4] this invention -- a law -- according to the method [PCR Protocols, Academic Press (1990), etc.], it carried out as follows using the half-quantitive PCR method. Moreover, the quantum of the transcript of the glyceraldehyde 3-phosphate dehydrogenase (glyceraldehyde-3-phosphate dehydrogenase;G3 PDH) considered to carry out the comparable manifestation in every cell was performed to coincidence, and it checked that it was practically equal to the conversion efficiency to a single strand cDNA from mRNA by the difference in the amount of mRNA(s) between cells, and the reverse transcriptase between samples.
[0194] mRNA of the Homo sapiens organ origin (the product made from Clontech: 3 caudate nucleus 2 brain 1 suprarenal gland) Four hippocampi, 5 substantia nigra, six thalami, the 7 kidney, the 8 pancreas, nine hypophyses, ten small intestines, Eleven bone marrow, 12 amygdalas, 13 cerebellums, 14 corpus callosa, 15 embryo brain, 16 embryo kidney, 17 embryo liver, 18 embryo lungs, the 19 heart, 20 liver, 21 lungs, 22 lymph gland, 23 mammary glands, 24 placentas, 25 prostate glands, 26 salivary glands, 27 skeletal muscle, 28 spines, The single strand cDNA was compounded using the cDNA composition kit (product made from SUPERSCRIPTTM Preamplification System; BRL) from 29 spleens, the 30 stomach, 31 testes, 32 thymus glands, the 33 thyroid, 34 tracheae, and 35 uteri. The single strand cDNA was compounded from mRNA of 1microg, and it diluted 240 times with water, and was used as mold of PCR. The synthetic DNA of a publication was used for the array numbers 16 and 17 based on the base sequence information from COL03279, the array numbers 18 and 19 based on the base sequence information from COL06772, the array numbers 20 and 21 based on the base sequence information from ADKA01604, and the array numbers 22 and 23 based on the base sequence information from ADSU00701 as a primer for PCR. The PCR reaction was performed according to the description using 10xGene Taq Universal Buffer and 2.5 mmol/ldNTP Mixture of NIPPON GENE Recombinant Taq DNA Polymerase (GeneTaq) and attachment. Thermal SAIKURA made from MJ RESERCH is used, and it is [degrees C / 94] 26 - 30 cycle ****** about the reaction for 2 minutes for 1 minute and at 72 degrees C for 30 seconds and in 60 degrees C. Reaction mixture was analyzed by agarose gel electrophoresis and ethidium-bromide dyeing.
[0195] A result is shown in drawing 1 -4. DNA of this invention accepted in each clone of COL03279, COL06772, ADKA01604, and ADSU00701 had discovered the difference of strength by each clone and each organ by all 35 which a certain thing examined sorts of organs.
[0196]
[Effect of the Invention] According to this invention, allergy, atopy, asthma, pollinosis, respiratory tract irritation, The disease accompanied by activation of unusual immunocytes, such as an autoimmune disease and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, traumatic brain injury, psoriasis, The disease accompanied by infection and inflammation of gout, various encephalomyelitis, congestive heart failure, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, rheumatoid arthritis, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunode-ficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve

cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:syste mic inflammatory responsesyndrome), Retrieval of remedies, such as adult respiratory distress syndrome (ARDS:adult respiratorydistress syndrome), The antisense DNA/RNA of DNA and this DNA which carries out the code of a useful polypeptide and this polypeptide to development, The antibody which recognizes the gene therapy using this DNA and this polypeptide, the activity rise alteration object of this polypeptide, the dominant negative variants of this polypeptide, and these directions can be offered.
[0197]
[Array table free text]
Explanation of an array number 11-artificial array: Composition RNA (oligo cap linker array)
Explanation of an array number 12-artificial array: Synthetic DNA (oligo dT primer array)
Explanation of an array number 13-artificial array: Synthetic DNA (sense primer array by the side of a five prime end)
Explanation of an array number 14-artificial array: Synthetic DNA (antisense primer array by the side of a three-dash terminal)
Explanation of an array number 15-artificial array (transcription factor NF-kappa junction sequence)
Explanation of an array number 16-artificial array: Synthetic DNA (synthetic primer array which considered organization manifestation distribution)
explanation: of an array number 17-artificial array -- explanation: of a synthetic DNA array number 18-artificial array -- explanation [of a synthetic DNA array number 19-artificial array]: -- explanation [of a synthetic DNA array number 20-artificial array]: -- explanation [of a synthetic DNA array number 21-artificial array]: -- explanation [of a synthetic DNA array number 22-artificial array]: -- explanation [of a synthetic DNA array number 23-artificial array]: -- a synthetic DNA [0198]
[Layout Table]
SEQUENCE LISTING <110> KYOWA HAKKO KOGYO CO. and LTD. -- <120> Novel polypeptide<130> H12-0641J5<140 <141>> -- < -- 160> 21<170> PatentIn Ver.2.1[0199 --] <210> 1<211> 780<212> PRT<213> Homo sapiens<400> 1Met Ala Ser Ala Glu Leu Gln-Gly-Lys-Tyr-Gln-Lys Leu Ala Gln Glu 1 5 10 15 Tyr Ser Lys Leu Arg-Ala-Gln-Asn-Gln Val Leu Lys Lys Gly-Val-Val 20 25 30 Asp Glu Gln Ala Asn Ser Ala Ala Leu Lys Glu Gln Leu Lys Met Lys 35 40 45 Asp Gln Ser Leu Arg Lys Leu Gln Gln Glu Met Asp Ser Leu Thr Phe 50 55 60 Arg Asn Leu Gln Leu Ala Lys Arg Val Glu Leu Leu Gln Asp Glu Leu 65 70 75 80 Ala Leu Se r GluPro Arg Gly Lys Lys Asn Lys Lys Ser Gly Glu Ser 85 90 95 Ser Ser Gln LeuSer Gln Glu Gln Lys Ser Val Phe Asp Glu Asp Leu 100 105 110 Gln Lys Lys IleGlu Glu Asn Glu Arg Leu His Ile Gln Phe Phe Glu 115 120 125 AlaAsp Glu Gln HisLys His Val Glu Ala Glu Leu Arg Ser Arg Leu 130 135 140 Ala Thr Leu Glu ThrGlu Ala Ala GlnHis Gln Ala Val Val Asp Gly 145 150 155 160 Leu Thr Arg Lys TyrMet Glu Thr Ile Glu Lys Leu Gln Asn Asp Lys 165 170 175 Ala Lys Leu Glu Val Lys Ser Gln Thr Leu Glu Lys Glu Ala Lys Glu 180 185 190 Cys Arg Leu Arg Thr Glu Glu CysGlnLeu Gln Leu Lys Thr Leu His 195 200 205 Glu Asp Leu Ser Gly Arg Leu Glu Glu Ser Leu Ser Il e Ile Asn Glu 210 215 220 Lys Val Pro Phe Asn Asp Thr Lys Tyr Ser Gln Tyr Asn Ala Leu Asn 225 230 235 240 Val Pro Leu His Asn Arg Arg His Gln Leu Lys Met Arg Asp Ile Ala 245 250 255 Gly Gln Ala Leu Ala Phe ValGln Asp Leu Val Thr Ala Leu Leu Asn 260 265270 Phe His Thr Tyr Thr Glu GlnArgIle Gln Ile Phe Pro Val Asp Ser 275 280 285 Ala Ile Asp Thr Ile Ser Pro Leu Asn Gln Lys Phe Ser Gln Tyr Leu 290 295 300 His Glu Asn Ala Ser Tyr Val Arg Pro Leu Glu Glu Gly Met Leu His 305 310 315 320 Leu Phe Glu Ser Ile Thr Glu Asp Thr Val Thr Val Leu Glu Thr-Thr 325 330 335 Val Lys Leu Lys Thr Phe Ser Glu His-Leu-Thr-Ser-Tyr-Ile-Cys-Phe 340 345 350 Leu Arg Lys Ile Leu Pro Tyr Gln Leu Lys Ser Leu Glu Glu-Glu-Cys 355 360365 Glu Ser Ser Leu Cys Thr Ser Ala Leu Arg Ala Arg Asn Leu Glu Leu 370 375 380 Ser Gln Asp Met Lys Lys Met Thr Ala Val Phe Glu Lys Leu Gln Thr 385 390 395 400 Tyr Ile Ala Leu Leu Ala Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu 405 410 415 Arg Thr Asn Tyr Ser Ser Val Leu Thr Asn Val Gly Ala Ala Leu His 420 425 430 Gly Phe His Asp Val Met Lys Asp Ile Ser Lys His Tyr Ser Gln Lys 435 440 445 Ala Ala

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LysLeu Cys Ser Ile Cys Lys Ala Met 180 185190 Glu Thr Trp Leu Ser Ala Asp Pro GlnHis Val Val Val Leu Tyr Cys 195 200 205 Lys Val Gly Gln Asp Leu Gly Phe Pro Gly Ala Trp Arg Phe Gln Val 210 215 220 Ser Leu Glu Leu Pro Asp Pro His Pro Cys Leu Ser Val Cys Gln Gly 225 230 235 240 Asn Lys Gly Lys Leu Gly Val Ile Val Ser Ala Tyr Met His Tyr Ser 245 250 255Lys Ile Ser Ala Gly 260 [0203]

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gag ctg agg agt cga ctg gcc act ctg gag aca gaa 607 His Val Glu Ala Glu Leu Arg Ser Arg Leu Ala Thr Leu Glu Thr Glu135 140 145 150gca gcc cag cac caa gct gtg gtt gac ggt ctc acc cgg aag tac atg 655 Ala Ala Gln His Gln Ala Val Val Asp Gly Leu Thr Arg Lys Tyr Met 155 160 165 gaa acc att gag aag ctg cag aac gac aag gct aaa cta gaa gtg aaa 703 Glu Thr Ile Glu Lys Leu Gln Asn Asp Lys Ala Lys Leu Glu Val Lys 170 175 180 tct cag-act-cta-gaa-aag gaa-gcc-aag-gaa-tgt cga ctt cga acg gaa 751 Ser Gln Thr Leu Glu Lys Glu Ala-Lys-Glu-Cys-Arg-Leu-Arg-Thr-Glu 185 190 195 gaa tgt caa tta cag-tta-aag-act-ctt cat gaa gat ttg tca ggt aga 799 Glu Cys Gln Leu Gln Leu Lys Thr Leu His Glu Asp Leu Ser Gly Arg 200 205 210 tta gag gaa tcc tta tca atcatc aat gaa aaa gta cct ttt aat gat 847 Leu Glu Glu Ser Leu Ser Ile Ile Asn Glu Lys Val Pro Phe Asn Asp215 220 225 230aca aaa tat agt cag tac aac gctctgaac gtt cca ctc cac aat agg 895 ThrLys Tyr Ser Gln Tyr Asn Ala Leu Asn Val Pro Leu His Asn Arg 235 240 245aga cac 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cca gta aga actaaa aaa cgt ttc aca 147 Thr Gln Ser Ala Ala IleVal Pro Val Arg Thr Lys Lys Arg Phe Thr 30 35 40 cct cct att tat caa cct aaa ttt aaa aca gaa aag gag ttt atgcaa 195 Pro Pro Ile Tyr Gln Pro Lys Phe Lys Thr Glu Lys Glu Phe Met Gln 45 50 55 cat gcc cgg aaa gca gga ttg gtt att cct cca gaa aaa tcg gac cgt 243 His Ala Arg LysAla Gly Leu Val Ile Pro Pro Glu Lys Ser Asp Arg 60 65 70 tcc ata cat ctg gcc tgt aca gct ggt ata ttt gat gcc tat gtt cct 291 Ser Ile His Leu AlaCys Thr Ala Gly Ile Phe Asp Ala Tyr Val Pro 75 80 85 90cct gag ggtgat gca cgc ata tca tct ctt tca aag gag gga ctg ata 339 Pro Glu Gly Asp Ala Arg Ile Ser Ser Leu Ser Lys Glu Gly Leu Ile 95 100 105 gag aga act gaacga atg aag aag act atg gca tca caa gtg tca atc 387 Glu ArgThr Glu Arg Met Lys Lys Thr Met Ala Ser Gln Val Ser Ile 110 115 120 cgg agg ata aaa gactat gat gcc aac tttaaaata aag gac ttc cct 435 Arg Arg Ile Lys Asp Tyr Asp Ala Asn Phe Lys Ile Lys Asp Phe Pro 125 130 135 gga aaa gctaag gat atc tttatt gaa gct cac ctt tgt cta aat aac 483 Gly Lys Ala Lys Asp Ile Phe Ile Glu Ala His Leu Cys Leu Asn Asn 140 145 150 tca gac cat gac cgactt cat accttg gta act gaa cac tgt ttt cca 531 Ser Asp His Asp Arg LeuHis Thr Leu Val Thr Glu His Cys Phe Pro155 160 165 170gac atg act tgg gac atc aaa tat aag acc gtc cgc tgg agc ttt gtg 579 AspMet Thr Trp Asp Ile Lys Tyr Lys Thr Val Arg Trp Ser Phe Val 175 180 185 gaa tct tta gag ccc tct cat gtt gtt caa gtt cgc tgt tca ag t atg 627 Glu Ser Leu Glu Pro Ser His Val Val Gln Val Arg Cys Ser Ser Met 190 195 200atg aac cag g gc aac gtg tac ggc cag-atc-acc-gta-cgc atg cac acc 675 Met-Asn-Gln-Gly-Asn Val Tyr Gly Gln Ile-Thr-Val-Arg-Met-His-Thr 205 210 215 cgg cag act ctg gcc-atc-tat-gac-cgg ttt ggc cgg ttg atg tat gga 723 Arg GlnThr Leu Ala Ile Tyr Asp Arg Phe Gly Arg Leu Met Tyr Gly 220 225 230 cag gaa gat gta ccc aag gat gtc ctg gag tat gtt gta ttc gaa aag 771 Gln Glu Asp Val Pro Lys Asp Val Leu Glu Tyr Val Val Phe Glu Lys235 240 245 250cag ttg aca aac ccc tat gga agc tgg aga atg cat acc aag atc gtt 819 Gln Leu Thr Asn Pro Tyr Gly Ser Trp Arg Met His Thr Lys Ile Val 255 260 265 ccc cca tgg gca ccc cct aag cag ccc atc ctt aag acg gtg atg atc 867 Pro Pro Trp Ala Pro Pro Lys Gln Pro Ile Leu Lys Thr Val Met Ile 270 275 280 cct ggc cct cag ctgaaa cca gaa gaa gaa tat gaa gag gca caa gga 915 Pro GlyPro Gln Leu Lys Pro Glu Glu Glu Tyr Glu Glu Ala Gln Gly 285 290 295 gag gcc cag aag cct cag cta gcc tgatgacaaaaatgacttct agggtgaagc 969 Glu Ala Gln Lys Pro Gln Leu Ala 300 305 ctgggtgatg aggctgctgg aagctttgaa gtctcccatt cccctcatgc tataaaaaga 1029 actacctttg ttctctccca tcctgctcag gtcttttcag cagtctcatc atcagcaacc 1089 atgactgatg actgggccct agcaggtggc aggtataaca tggccatgga cactcttctt 1149ttttaaattttatgtctag(s)c ttctgagtct agatgaaaga cagtatgttt cagagaacat 1209 tggatatcag tttttcccac agcagggact gtgagagaca accagcagca tcctctttgt 1269 aatcacaggg cagggatcag agtttgaaat gaaatgttgt cagggtgttg gaaaaatttt 1329 ggtgagttct gcacatttcc cctggttcag gctgggcatg gaccagcctt cagatggcag 1389 aagtggaaga tgagcctact tgtgagcgat gtgactttaa ggaaatgagg actggggaag 1449 aataattagt gtttataaga catttaagag gccctttttc atatactgac tcactgatga 1509 atcagcattt gcattttatg gaaaaatata aatgcaaaga aataatttaa aaaaaaaaaa 1569aaaaa 1574 [0207]
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[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the result of investigating the amount of manifestations of the COL03279 imprint object in 35 sorts of human tissues (organ) using ** and the PCR method.
[Drawing 2] It is the result of investigating the amount of manifestations of the COL06772 imprint object in 35 sorts of human tissues (organ) using ** and the PCR method.
[Drawing 3] It is the result of investigating the amount of manifestations of the ADKA01604 imprint object in 35 sorts of human tissues (organ) using ** and the PCR method.
[Drawing 4] It is the result of investigating the amount of manifestations of the ADSU00701 imprint object in 35 sorts of human tissues (organ) using ** and the PCR method.
[Description of Notations]
The figure of a publication in a complete diagram and the alphabet are as follows.
A suprarenal gland, 02:brain, 03:caudate nucleus, 04:hippocampus, 05:substantia nigra, 06 : 01: A thalamus, 07 : The kidney, 08:pancreas, 09 hypophyses, 10:small intestine, 11:bone marrow, 12 : An amygdala, 13:cerebellum, 14:corpus callosum, 15:embryo brain, 16:embryo kidney, 17: Embryo liver, 18:embryo lungs, 19:heart, 20:liver, 21 : Lungs, 22: -- lymph gland and 23: -- a mammary gland, 24:placenta, 25:prostate gland, 26:salivary glands, 27:skeletal muscle, and 28: -- a spine, 29:spleen, 30:stomach, 31:testis, 32:thymus gland, and 33: -- the thyroid, 34:trachea, 35:uterus, Pr:plasmid, and M:molecular weight marker

[Translation done.]